BERGEY'S MANUAL OF

Systematic Bacteriology

Volume 1

NOEL R. KRIEG
EDITOR, VOLUME 1
JOHN G. HOLT
EDITOR-IN-CHIEF

EDITORIAL BOARD
R. G. E. Murray, Chairman, **Don J. Brenner**
Marvin P. Bryant, John G. Holt, Noel R. Krieg
James W. Moulder, Norbert Pfennig
Peter H. A. Sneath, James T. Staley

WITH CONTRIBUTIONS FROM
124 COLLEAGUES

WILLIAMS & WILKINS
Baltimore/London

SECTION 5

Facultatively Anaerobic Gram-Negative Rods

Table 5.1.
Some differential characteristics of the families of Section 5[a]

Characteristics	*Enterobacteriaceae* (p. 408)	*Vibrionaceae* (p. 516)	*Pasteurellaceae* (p. 550)
Cell diameter, μm	0.3–1.5	0.3–1.3	0.2–0.3
Straight rods	+	D	+
Curved rods	–	D	–
Motility	D	+[b]	–
Flagellar arrangement (liquid media):			
Polar	–	+	
Lateral	+[c]	–	
Oxidase test	–	+[b]	+[b]
Na^+ required or stimulatory for growth	–	D	–
Contain enterobacterial common antigen	+[d]	–[e]	–
Cells contain menaquinones[f]	D	D	–
Parasitic on mammals and birds	D	–[b]	+
Heme and/or nicotinamide adenine dinucleotide required for growth	–	–	D
Plant pathogenicity	D	–	–
Organic nitrogen sources required	–[b]	–[b]	+

[a] Symbols: see standard definitions.
[b] A few exceptions may occur.
[c] Except *Tatumella*, which may have polar, subpolar or lateral flagella.
[d] *Erwinia chrysanthemi* does not contain the antigen.
[e] *Pleisomonas shigelloides* contains the antigen.
[f] *Pasteurellaceae* do contain demethylmenaquinones but not menaquinones; ubiquinones may or may not be produced. *Enterobacteriaceae* and *Vibrionaceae* may contain menaquinones, demethylmenaquinones and ubiquinones.

FAMILY I. ENTEROBACTERIACEAE RAHN 1937, Nom. fam. cons. Opin. 15, Jud. Comm. 1958, 73; Ewing, Farmer, and Brenner 1980, 674; Judicial Commission 1981, 104.

DON J. BRENNER

En.te.ro.bac.te.ri.a'ce.ae. M.L. n. *enterobacterium* an intestinal bacterium; *-aceae* ending to denote a family; M.L. fem. pl. n. *Enterobacteriaceae* the family of the enterobacteria. Rahn's original derivation is not certain. It may have come from his genus *Enterobacter*, or may have come from the root enterobacterium.

Gram-negative straight rods, 0.3–1.0 × 1.0–6.0 μm; motile by peritrichous flagella, except for *Tatumella*, or nonmotile. Do not form endospores or microcysts; not acid-fast. Grow in the presence and absence of oxygen. Grow well on peptone, meat extract, and usually MacConkey's media. Some grow on D-glucose as the sole source of carbon, others require vitamins and/or amino acids. Chemoorganotrophic; respiratory and fermentative metabolism. Not halophilic. Acid and often visible gas is produced during fermentation of D-glucose, other carbohydrates and polyhydroxyl alcohols. Catalase-positive except for *Shigella dysenteriae* 0 group 1 and *Xenorhabdus nematophilus*; oxidase

negative. Nitrate reduced to nitrite except by some strains of *Erwinia* and *Yersinia*. G + C content of DNA is 38–60 mol% (T_m, Bd).

DNAs from species within most genera are at least 20% related to one another and to *Escherichia coli*, the type species of the family. Notable exceptions are species of *Yersinia*, *Proteus*, *Providencia*, *Hafnia* and *Edwardsiella*, whose DNAs are 10–20% related to those of species from other genera.

Except for *Erwinia chrysanthemi* (Le Minor et al., 1972) all species tested contain the enterobacterial common antigen (Kunin, 1963; Kunin et al., 1962; Whang and Neter, 1962; Vosti et al., 1964; Le Minor et al., 1972).

Type genus: Escherichia Castellani and Chalmers 1919, 941. Designated type genus Opin. 15, Jud. Comm. 1958, 73.

Further Comments

Circumscription. The definition circumscribes a large biochemically and genetically related group that shows substantial heterogeneity in its ecology, host range and pathogenic potential for man, animals, insects and plants. The delimitation of *Enterobacteriaceae* from members of other families seems complete, except as mentioned below; however, systematic studies have rarely been done.

The genera *Vibrio*, *Photobacterium*, *Aeromonas* and *Plesiomonas* are oxidase-positive and have polar flagella when grown in liquid media—characteristics which distinguish them from *Enterobacteriaceae*. However, at least two *Vibrio* species (*V. metschnikovii* and *V. gazogenes*) are oxidase-negative; strains of other species are oxidase-negative or weakly positive; and, under certain conditions (often on solid media), members of these genera produce peritrichous flagella. *P. shigelloides* is the only member of *Vibrionaceae* to contain the enterobacterial common antigen (Le Minor et al., 1972). Some *Aeromonas* strains show higher DNA relatedness to *E. coli*, the type species of *Enterobacteriaceae*, than that seen with several genera within the family. In fact, *Enterobacteriaceae* and *Vibrionaceae* have been treated as a superfamily. Nonetheless, the functional distinction between *Enterobacteriaceae* and *Vibrionaceae* is extremely useful and essentially exclusive. If one imagines an evolutionary continuum from a common ancestor, it is not surprising to find gray areas or areas of overlap between families.

Subdivision of the family. In previous editions of *Bergey's Manual* the family was divided into tribes largely on the basis of fermentation of D-glucose by the mixed acid pathway (positive methyl red reaction) or by the 2,3-butanediol pathway (positive Voges-Proskauer reaction), urease and KCN. The use of tribes is of no diagnostic significance and of questionable taxonomic significance. The latter contention is supported by the fact that the tribes listed and the genera included in various tribes changed markedly between Bergey's seventh and eighth editions. In the seventh edition, the tribe *Escherichieae* contained the genera *Escherichia*, *Enterobacter*, and *Klebsiella*, but the genera *Salmonella* and *Shigella* were in the tribe *Salmonelleae*. In the eighth edition the tribe *Salmonelleae* was deleted, *Klebsiella* was placed in the tribe *Klebsielleae*, and *Salmonella* and *Shigella* were transferred to *Escherichieae*. A further problem with the tribe concept is that the only tribes appearing on the Approved Lists of Bacterial Names (Skerman et al., 1980) are *Erwinieae*, *Escherichieae*, *Proteeae*, *Salmonelleae* and *Serratieae*. The tribe concept, therefore, is not used in the present edition of the *Manual*. Although far from perfect, arbitrary DNA relatedness groupings (Figs. 5.1 and 5.2) approximate evolutionary divergence within the genera.

Further notes. Enterobacteriaceae are distributed worldwide. They are found in soil, water, fruits, vegetables, grains, flowering plants and trees, and in animals from insects to man. Their medical and economic importance, as well as their rapid generation time, ability to grow on defined media, and ease of genetic manipulation have made them the objects of intense laboratory study.

Many species are of considerable economic importance. Erwiniae cause blight, wilt and soft-rot disease in corn, potatoes, pineapples and many other crops, often destroying substantial amounts of the crops (Starr and Chatterjee, 1972). The commercial and tropical fish industries are severely affected by the diseases caused by *Yersinia ruckeri* and species of *Edwardsiella* (Ewing et al., 1978; Shotts and Snieszo, 1976).

Salmonellosis in poultry is a worldwide problem, both for poultry farmers and as a vehicle for human disease (Williams, 1965; Von Rockel, 1965; Hall, 1965). Stillbirths and wool damage in sheep are usually caused by salmonellae (Jensen, 1974). *Escherichia coli* strains that have the K99 colonization factor and produce enterotoxin are primarily responsible for diarrhea in lambs. Enterotoxigenic strains of *E. coli* containing specific colonization factors are also responsible for highly fatal diarrhea in piglets and calves (Bruner and Gillespie, 1973). Klebsiellae and *Citrobacter freundii* cause bovine mastitis. Salmonellosis is also common in pigs, cows, horses, dogs and cats (Barnes and Sorensen, 1975; Ewing, 1969).

Numerous other animal infections are caused by *Enterobacteriaceae*. A few examples are sexually transmitted uterine infections in horses caused by a limited number of capsular types of *Klebiella pneumoniae*; infections in snakes, turtles and lizards caused by salmonellae; diarrheal and septicemic infection in rabbits, other rodents and minks caused by yersiniae; and shigellosis in monkeys. Salmonellae remain the most frequently encountered etiologic agents of food-borne disease.

Until the 1940s only *Salmonella* (including *Arizona*) and *Shigella* were considered as gastrointestinal pathogens. It is now well established that *E. coli* is a significant cause of diarrheal illness both in infants and adults in many areas of the world. Invasive and enterotoxigenic strains of *Yersinia enterocolitica*, apparently restricted to certain serovars ("serotypes"; see preface to the *Manual*, p. xiii), cause diarrhea and mesentery lymphadenitis. Enterotoxigenic strains of *Klebsiella pneumoniae* have frequently been isolated from patients with tropical sprue (Klipstein et al., 1973). Enterotoxin production has also been reported for an occasional strain of *Enterobacter*. Since the enterotoxin genes in *E. coli* are on transmittable plasmids (Smith and Halls, 1968; Gyles et al., 1974), it would not be surprising to find enterotoxin-producing strains in other species of *Enterobacteriaceae*.

Species of *Enterobacteriaceae* not normally associated with diarrheal disease are often referred to as opportunistic pathogens. Most of these species can cause a variety of extraintestinal infections. The compromised host (for example, the malnourished, diabetic, immunosuppressed, catheterized, burn, cancer, respiratory or elderly patient) is vulnerable to nosocomial infections caused by opportunistic pathogens. *Enterobacteriaceae* have been responsible for about 50% of nosocomial infections in the United States (Center for Disease Control, 1977). These infections were most frequently caused by *E. coli*, *Klebsiella*, *Enterobacter*, *Proteus*, *Providencia*, and *Serratia marcescens*.

Compared with the eighth edition of *Bergey's Manual*, the present volume contains many nomenclatural changes, several new genera, and many new species. Also, in contrast to the eighth edition, type strains have been designated for all species. The main reasons for these changes are: (a) a conservative approach to *Enterobacteriaceae* was taken in the eighth edition, and therefore, descriptions of several known species and several nomenclatural proposals were omitted or only mentioned informally in the text; (b) contributions to the chapter on *Enterobacteriaceae* in the eighth edition were completed before 1970, so more than a decade has elapsed since the last edition; (c) environmental and animal studies have uncovered new species from water, insects, nematodes, plants, fish and small animals; and (d) data from DNA relatedness studies have provided criteria for a species definition that can be used to determine whether any new or biochemically atypical group represents a new species or a biogroup within an existing species.

In Table 5.2 the current classification is compared with that in the eighth edition. There are a number of species for which nomenclatural synonyms exist (Table 5.2). Some of these, *S. paratyphi* B, *S. paratyphi* C, *S. daressalaam*, *A. hinshawii*, are not on the Approved Lists of Bacterial Names (Approved Lists) (Skerman et al., 1980). The *Salmonella* serovar names have no standing in nomenclature, but continue to be used as an extremely useful form of communication. *Arizona hinshawii* was included on the final list of species sent from the

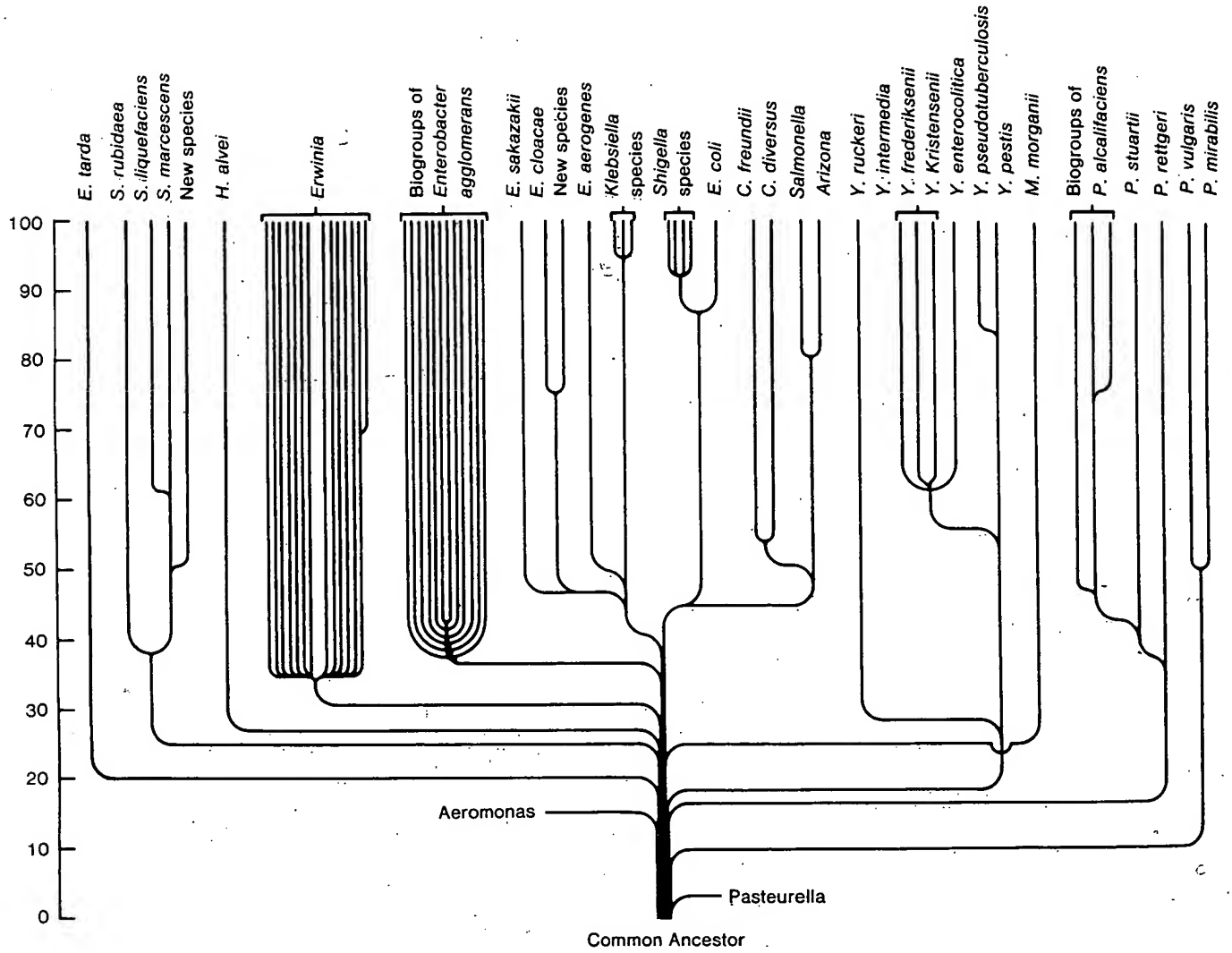

Figure 5.1. Divergence of *Enterobacteriaceae*. The *ordinate* is percentage of relatedness. This figure is a simplified attempt to depict relatedness of each species of enterobacteria to all other species. It assumes a common ancestor from which all of the organisms have diverged. The horizontal branches depict the degree of relatedness of the group of organisms to all organisms that have not yet branched. For example, *E. tarda* is ~20% related to all organisms except *Aeromonas*, *Proteus*, *Providencia* and *Pasteurella*; *Citrobacter* species are ~45% related to all species above them and *C. diversus* and *C. freundii* were speciated at a point in time such that they are now 50% related.

International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Enterobacteriaceae* (*Enterobacteriaceae* Subcommittee), but was omitted from the Approved Lists. Therefore, *Arizona hinshawii* no longer has standing in the literature, although it is widely used in the United States and elsewhere. It remains to be seen whether *Arizona hinshawii* will be reproposed (see section on "Taxonomic and Nomenclatural Problems" below).

Taxonomic and Nomenclatural Problems

Escherichia *and* Shigella. The organism named *Escherichia adecarboxylata* (Leclerc, 1962) appears on the Approved Lists. It is negative in decarboxylase reactions and positive in reactions for KCN, malonate, cellobiose, and often urea. It may belong in the *Erwinia herbicola-Erwinia stewartii-Erwinia uredovora-Enterobacter agglomerans (Erwinia-E. agglomerans)* complex rather than in the genus *Escherchia* (Bascomb et al., 1971; Ewing and Fife, 1972). DNA relatedness studies will be necessary to resolve the status of *E. adecarboxylata*.

The four species of *Shigella* and *E. coli* are a single species on the basis of DNA relatedness (Brenner et al., 1972, 1973). *Shigella* and *E. coli* strains are often extremely difficult to separate biochemically because there are aerogenic (gas-producing) shigellae and lactose-negative, anaerogenic, nonmotile *E. coli*. *E. coli* strains can cause a dysentery-like diarrhea, so pathogenicity does not provide definitive separation. Shigellae are actually metabolically inactive biogroups of *E. coli*. It is taxonomically difficult to justify separate genera or even separate species status for these organisms. They remain separate species because of the ease of communication these names provide in medical microbiology and because of the resistance and confusion that would be caused by reclassification. (However, the original usage implied that shigellae were pathogenic and that *E. coli* was not; this is certainly not true). Nonetheless, *Vibrio cholerae* was similarly reclassified, and it is now proposed that *Yersinia pestis* be taxonomically, but not practically, considered as a subspecies of *Y. pseudotuberculosis* (see *Yersinia* below). Perhaps a similar future recommendation will be made for *E. coli* and *Shigella*—namely, that they be a single species with five subgroups for taxonomic purposes, but that they continue to be treated and written as separate genera or species.

Edwardsiella. The name *Edwardsiella anguillimortifera* was proposed as a senior synonym for *Edwardsiella tarda* (Sakazaki and Tamura, 1975). No available strains correspond to the description of

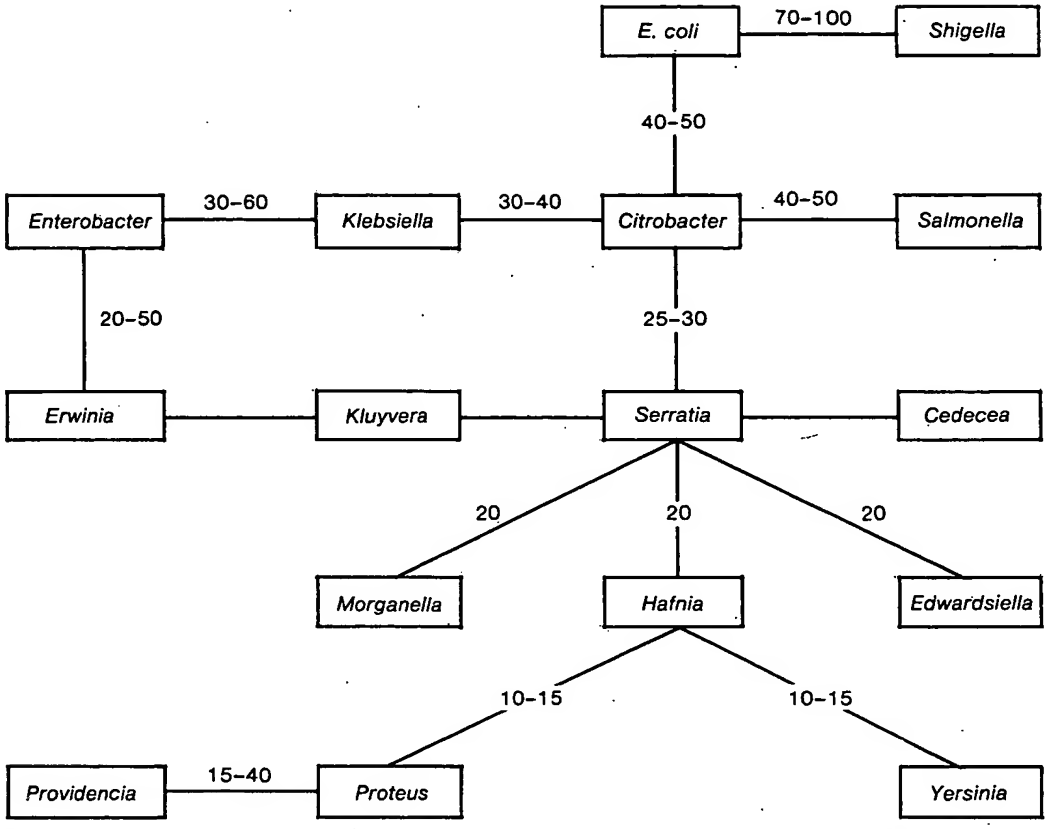

Figure 5.2. DNA relatedness among *Enterobacteriaceae*. The numbers represent the approximate percentage of relatedness.

E. anguillimortifera (which differs from the description of *E. tarda*). This problem is discussed in the chapter on *Edwardsiella*.

Citrobacter. DNA relatedness studies leave little doubt that *Levinea malonatica (Citrobacter diversus)* and *Levinea amalonatica* belong with *Citrobacter freundii* in the genus *Citrobacter* (Crosa et al., 1974), rather than in the genus *Levinea* as proposed by Young et al. (1971). Both *Levinea* species were contained as biogroups of "*Citrobacter intermedius*" in the eighth edition of *Bergey's Manual*. *C. intermedius* did not have a type strain and does not appear on the Approved Lists. The specific epithets *diversus*, *koseri* and *malonatica* are admittedly synonyms, are all validly published, and all appear on the Approved Lists. Both *C. diversus* and *C. koseri* (Frederiksen, 1970) have priority over the subjective synonym *L. malonatica*. *C. diversus* dates back to 1928, whereas *C. koseri* was named in 1970; however, questions have been raised about the correspondence of *C. diversus* to the original description of this organism (Holmes et al., 1974). *Levinea amalonatica* became *Citrobacter amalonaticus* when it was transferred to the genus *Citrobacter* (Brenner and Farmer, 1981; Brenner et al., 1977). This three-species concept is available for those who do not accept *Levinea* as a separate genus (Farmer, 1981).

Salmonella. In the eighth edition of *Bergey's Manual*, Le Minor and Rohde (1974) stated that "scientifically none of the present methods of nomenclature of *Salmonella* is satisfactory" and that "the International *Enterobacteriaceae* Subcommittee has not given clear guidance on the naming of the different types." Unfortunately, these statements remain just as true today as they were more than a decade ago. The use of "species" names for *Salmonella* serovars is extremely useful in many fields. As long as these serovar names are not *taxonomically* equated with species, this practice should be encouraged as stated in the chapter on *Salmonella*. It is the taxonomic treatment of salmonellae that is untenable. DNA relatedness data have shown that representative strains of biotypically typical *Salmonella* serovars (subgenus I), biochemically atypical *Salmonella* serovars (subgenera II and IV) and *S. arizonae* (subgenus III) belong to a single genetic species (Crosa et al., 1973; Stoleru et al., 1976). Five subgroups were distinguishable within this single genetic *Salmonella* species. They corresponded to *Salmonella* subgenera I; II; III (*S. arizonae* with monophasic flagellar antigens); III (*S. arizonae* with diphasic flagellar antigens); and IV.

The logical classification of salmonellae should therefore be as a single diverse species with five subspecies. An acceptable name would have to be proposed for the single species, and names, as well as type strains, would have to be proposed for each subspecies. Since *S. houtenae* was the type for subgenus IV and *S. salamae* was the type for subgenus II and type strains were designated for each of these (they are not on the Approved Lists), they could be reproposed and serve for two of the subspecies. *S. arizonae* has a type strain with monphasic flagellar antigens which could serve for the monophasic subspecies of what is now subgenus III. One possibility for the subspecies with diphasic flagella would be subspecies "*hinshawii*." A type strain would have to be designated. The remaining problem would be to designate a name and a type species for the subspecies corresponding to subgenus I. This would also be the species name. The oldest serovar appears to be *S. typhi*, which entered the literature in 1886; however, naming the single species after any existing serovar, especially *S. typhi*, would cause massive confusion and would be unwise. Perhaps this dilemma could be solved by following the suggestion of Kauffmann and Edwards (1952) to designate "*S. enterica*" as the single species name. The type strain might then be a strain of serovar *S. typhi*, or, since *S. typhi* is not biochemically typical, a strain of *S. typhimurium*, the most frequently occurring serovar, could serve. Another alternative for the type strain could be a rough strain.

In medial bacteriology Latin binomials would be used for serovars from subgenus I and for the names serovars in subgenera II and IV. Unnamed serovars in subgenera II, III and IV could be listed by

Table 5.2.
Comparison of current classification with that in the eighth edition of Bergey's Manual

Current Classification	Synonyms[a]	*Bergey's* 8th
Escherichia coli		*E. coli*
E. blattae		NL[b]
Shigella dysenteriae		*S. dysenteriae*
S. flexneri		*S. flexneri*
S. boydii		*S. boydii*
S. sonnei		*S. sonnei*
Edwardsiella tarda	*E. anguillimortifera*	*E. tarda*
E. ictaluri		NL
E. hoshinae		NL
Citrobacter freundii		*C. freundii*
C. diversus	*Levinea malonatica, C. koseri*	*C. intermedius* biogroup b
C. amalonaticus	*Levinea amalonatica*	*C. intermedius* biogroup a
Salmonella choleraesuis		*S. cholerae-suis*
S. hirschfeldii[c]	*S. paratyphi C*[c]	*S. hirschfeldii*
S. typhi		*S. typhi*
S. paratyphi A[c]		*S. paratyphi* A
S. schottmuelleri	*S. paratyphi B*[c]	*S. schottmuelleri*
S. typhimurium		*S. typhimurium*
S. enteritidis		*S. enteritidis*
S. gallinarum[c]		*S. gallinarum*
S. salamae[c]	*S. daressalaam*[c]	*S. salamae*
S. arizonae	*Arizona hinshawii*[c]	*S. arizonae*
S. houtenae[c]		*S. houtenae*
Klebsiella pneumoniae subsp. *pneumoniae*		*K. pneumoniae*
K. pneumoniae subsp. *ozaenae*[c]		*K. ozaenae*
K. pneumoniae subsp. *rhinoscleromatis*[c]		*K. rhinoscleromatis*
K. oxytoca		K. pneumoniae, indole-positive biogroup
K. planticola		NL
K. terrigena		NL
Enterobacter cloacae		*E. cloacae*
E. aerogenes	*Klebsiella mobilis*	*E. aerogenes*
E. agglomerans		*Erwinia herbicola*, *Erwinia stewartii*, and *Erwinia uredovora*
E. gergoviae		NL
E. amnigenus		NL
E. sakazakii		yellow-pigmented *E. cloacae*
E. intermedium		NL
Hafnia alvei		*H. alvei*
Serratia marcescens		*S. marcescens*
S. liquefaciens		NL
S. rubidaea	*S. marinorubra*	NL
S. plymuthica		biogroup of *S. marcescens*
S. proteamaculans		NL
S. odorifera		NL
S. fonticola		NL
S. ficaria		NL
Proteus vulgaris		*P. vulgaris*
P. mirabilis		*P. mirabilis*
P. myxofaciens		NL
Providencia alcalifaciens		*Proteus inconstans* biogroup A
P. stuartii		*Proteus inconstans* biogroup B
P. rettgeri		*Proteus rettgeri*
Morganella morganii		*Proteus morganii*
Yersinia pseudotuberculosis		*Y. pseudotuberculosis*
Y. pestis		*Y. pestis*
Y. enterocolitica		*Y. enterocolitica*
Y. ruckeri		NL
Y. intermedia		biogroup of *Y. enterocolitica*
Y. frederiksenii		biogroup of *Y. enterocolitica*
Y. kristensenii		biogroup of *Y. enterocolitica*
Erwinia amylovora		*E. amylovora*
E. salicis		*E. salicis*

Table 5.2—*continued*

Current Classification	Synonyms[a]	*Bergey's* 8th
E. tracheiphila		*E. tracheiphila*
E. nigrifluens		*E. nigrifluens*
E. quercina		*E. quercina*
E. rubrifaciens		*E. rubrifaciens*
E. herbicola	*Enterobacter agglomerans*	*E. herbicola*
E. stewartii	*Enterobacter agglomerans*	*E. stewartii*
E. uredovora	*Enterobacter agglomerans*	*E. uredovora*
E. carotovora	*Pectobacterium carotovorum*	*E. carotovora*
E. chrysanthemi	*Pectobacterium chrysanthemi*	*E. chrysanthemi*
E. cypripedii	*Pectobacterium cypripedii*	*E. crypripedii*
E. rhapontici	*Pectobacterium rhapontici*	*E. rhapontici*
E. carnegieana	*Pectobacterium carnegieana*	NL
E. mallotivora		NL
Obesumbacterium proteus		NL
Kluyvera ascorbata		NL
K. cryocrescens		NL
Cedecea lapagei		NL
C. davisae		NL
Tatumella ptyseos		NL
Xenorhabdus nematophilus		NL
X. luminescens		NL
Rahnella aquatilis		NL

[a] Synonyms are of several types: objective, subjective, challenged, those that are on the Approved Lists of Bacterial Names (Skerman et al., 1980), and those which no longer have standing in the literature. See text for explanation.

[b] NL, not listed.

[c] Not on Approved Lists of Bacterial Names (Skerman et al., 1980) and have no current standing in nomenclature.

subspecies names followed by the antigenic formula (e.g., *S. salamae* 40:b:-; *S. houtenae* 43:z_{29}:-; *S. arizonae* 5:1,6,7:-; "*S. hinshawii*" 1,4:33:31).

The *Enterobacteriaceae* Subcommittee must reconsider the problems in classification of salmonellae and recommend a solution that is both consistent with the taxonomic data and that will serve the needs of all microbiologists. The suggestions given above are neither original nor formal proposals. They are meant to reiterate the problem and to exemplify a possible solution.

Klebsiella. *K. pneumoniae, K. ozaenae* and *K. rhinoscleromatis* are bio-sero-pathogroups of one genetic species (Brenner et al., 1972). As with shigellae, they have traditionally been separated because of their medical interest, but it would be more accurate taxonomically to treat them as subspecies of *K. pneumoniae*, and this has been done in the present edition of the *Manual*. For medical purposes, however, one would expect them to continue to be reported as if they were separate species. *Klebsiella mobilis* appears on the Approved Lists. It is an objective synonym for *Enterobacter aerogenes* and will be discussed with *Enterobacter*. "*Klebsiella aerogenes*," "*Klebsiella edwardsii*," and "*Klebsiella atlantae*" are usually considered as biogroups of *K. pneumoniae*. They do not have standing in the literature, but are used clinically in Great Britain and in other parts of the former British Commonwealth. Their descriptions are given by Cowan (1974). Strains of *Klebsiella pneumoniae* that are motile and Voges-Proskauer-negative were recently reported by Ferragut and Leclerc (1978). This finding cannot be considered definitive as these strains are no longer motile (D. Izard, personal communication).

Enterobacter. *Erwinia dissolvens* and *Erwinia nimipressuralis*, two species formerly thought to be atypical erwiniae, are closely related to the genus *Enterobacter* both biochemically and by DNA hybridization (Dye, 1969; Steigerwalt et al., 1975). Both are most closely related to *Enterobacter cloacae* and belong in the genus *Enterobacter* either as biogroups of *E. cloacae* or as separate species. Very few strains have been studied; therefore, no formal proposals have been made for their classification (Steigerwalt et al., 1975). A third species, *Erwinia cancerogena*, is also thought to belong to *Enterobacter* (Lelliott, 1974).

Enterobacter aerogenes is biochemically and by DNA hybridization (Brenner et al., 1972; Steigerwalt et al., 1975) equally or more related to klebsiellae than to other *Enterobacter* species. For this reason, there have been attempts to place it in the genus *Klebsiella*. *Klebsiella mobilis* was proposed by Bascomb et al. (1971). *K. mobilis* and *E. aerogenes* have the same type strain and are, by definition, objective synonyms. If *E. aerogenes* is transferred to the genus *Klebsiella*, it would become the new combination "*K. aerogenes*." This name would be most confusing, however, because it formerly was used for some strains of *Klebsiella pneumoniae* (Cowan et al., 1960). At present "*K. aerogenes*" has no standing in the literature (it is not on the Approved Lists), and to repropose it for a different group would only cause confusion. *K. mobilis* is, in a sense, confusing, since there are nonmotile strains of *E. aerogenes*. The use of *K. mobilis* would also cause confusion due to the loss of the epithet "*aerogenes*," which is now well accepted. A decision must be made as to whether the classification of *Enterobacter aerogenes* should remain status quo, whether it should be transferred to *Klebsiella* as *K. mobilis*, or whether it should be transferred to *Klebsiella* with a new species name.

Enterobacter agglomerans was proposed by Ewing and Fife (1971, 1972) for an organism(s) that had been in the literature previously in *Erwinia*, under a large number of species names. They argued that all of the previous names, including *Erwinia herbicola*, *Erwinia stewartii* and *Erwinia uredovora*, which were listed in the eighth edition and the present volume of *Bergey's Manual*, were synonyms and junior to the description of *Bacillus agglomerans*. They felt that biochemically the organism belonged in the genus *Enterobacter* rather than in the genus *Erwinia*. They further stated that this species, represented by seven anaerogenic and four aerogenic biogroups, might subsequently be transferred to a new genus which, if necessary, could be subdivided into more than one species. DNA hybridization studies (D.J. Brenner, J. Leete, G.R. Fanning, R.G. Steigerwalt and M. Krichevsky, unpublished data) indicate that neither the three-species *Erwinia* scheme nor the 11-biogroup scheme agree with DNA relatedness data. There appear to be 10 or more DNA relatedness groups within the *Erwinia-E. agglomerans* complex. The type strain for *E. agglomerans* is different from the

Table 5.3.
Biochemical identification of **Enterobacteriaceae**[a]

Characteristics	*Cedecea davisae*	*Cedecea lapagei*	*Citrobacter amalonaticus*	*Citrobacter diversus*	*Citrobacter freundii*	*Edwardsiella hoshinae*	*Edwardsiella ictaluri*	*Edwardsiella tarda*	*Edwardsiella tarda* biogroup 1	*Enterobacter aerogenes*	*Enterobacter agglomerans*	*Enterobacter cloacae*	*Enterobacter gergoviae*	*Enterobacter intermedium*	*Enterobacter sakazakii*	*Escherichia adecarboxylata*	*Escherichia blattae*	*Escherichia coli*	*Escherichia coli*, inactive	*Hafnia alvei*
Indole production	−	−	+	+	−	[−]	−	+	+	−	[−]	−	−	−	[−]	+	−	+	[+]	−
Methyl red	+	d	+	+	+	+	−	+	+	−	d	−	d	d	[−]	+	+	+	+	d
Voges-Proskauer	[+]	+	−	−	−	−	−	−	−	+	d	+	+	+	+	−	−	−	−	d
Citrate, Simmons'	+	[+]	[+]	+	+	−	−	−	−	+	d	+	+	+	+	−	d	−	−	−
Hydrogen sulfide on TSI	−	−	−	−	[+]	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
Urease, Christensen's	−	−	[+]	[+]	d	−	−	−	−	−	[−]	d	+	d	−	d	−	−	−	−
Phenylalanine deaminase	−	−	−	−	−	−	−	−	−	−	[−]	−	−	−	d	−	−	−	−	−
Lysine decarboxylase	−	−	−	−	−	+	+	+	+	+	−	−	+	−	−	−	+	[+]	d	+
Arginine dihydrolase	d	[+]	[+]	d	d	−	−	−	−	−	−	+	−	d	+	−	−	[−]	−	−
Ornithine decarboxylase	+	−	+	+	[−]	+	d	+	+	+	−	+	+	+	+	−	+	d	[−]	+
Motility	+	[+]	+	+	+	+	−	+	+	+	[+]	+	+	+	+	+	−	[+]	−	+
Gelatin liquefaction at 22°C	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	d	−	−	−	−
KCN, growth in	[+]	d	+	−	+	−	−	−	−	+	d	+	−	+	+	+	−	−	−	+
Malonate utilization	[+]	+	[−]	+	[−]	+	−	−	−	+	d	[+]	+	+	[−]	d	+	−	−	d
D-Glucose, acid production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Glucose, gas production	[+]	+	+	+	+	d	d	+	d	+	[−]	+	+	+	+	+	+	+	−	+
Lactose	−	d	d	d	d	−	−	−	−	+	d	+	d	+	+	+	−	+	[−]	−
Sucrose	+	−	[−]	[−]	d	+	−	−	+	+	[+]	+	+	d	+	+	−	d	[−]	−
D-Mannitol	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	−	+	+	+
Dulcitol	−	−	−	d	d	−	−	−	−	−	[−]	[−]	−	d	−	+	−	d	d	−
Salicin	+	+	d	[−]	−	d	−	−	−	+	d	[+]	+	+	+	+	−	d	−	[−]
D-Adonitol	−	−	−	+	−	−	−	−	−	+	−	[−]	−	−	−	+	−	−	−	−
myo-Inositol	−	−	−	−	−	−	−	−	−	+	[−]	[−]	−	−	[+]	−	−	−	−	−
D-Sorbitol	−	−	+	+	+	−	−	−	−	+	d	+	−	+	−	−	−	+	[+]	−
L-Arabinose	−	−	+	+	+	[−]	−	−	+	+	+	+	+	+	+	+	+	+	[+]	+
Raffinose	−	−	−	−	d	−	−	−	−	+	d	+	+	+	+	+	−	d	[−]	−
L-Rhamnose	−	−	+	+	+	−	−	−	−	+	[+]	+	+	+	+	+	+	[+]	d	+
Maltose	+	+	+	+	+	+	+	+	+	+	[+]	+	+	+	+	+	+	+	[+]	+
D-Xylose	+	−	+	+	+	−	−	−	−	+	+	+	+	+	+	+	+	+	d	+
Trehalose	+	+	+	+	+	+	−	−	−	+	+	+	+	+	+	+	[+]	+	+	+
Cellobiose	+	+	+	+	d	−	−	−	−	+	d	+	+	+	+	+	−	−	−	[−]
α-Methyl-D-glucoside	−	−	−	d	−	−	−	−	−	+	−	[+]	−	d	+	−	−	−	−	−
Esculin hydrolysis	d	+	−	−	−	−	−	−	−	+	d	d	+	d	+	+	−	d	−	−
Melibiose	−	−	[−]	−	d	−	−	−	−	+	d	+	+	d	+	+	−	[+]	d	−
D-Arabitol	+	+	−	+	−	−	−	−	−	+	d	[−]	+	−	−	+	−	−	−	−
Mucate	−	−	+	+	+	−	−	−	−	+	d	[+]	−	d	−	+	d	+	d	−
Lipase, corn oil	[+]	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Deoxyribonuclease at 25°C	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
$NO_3^- \rightarrow NO_2^-$	+	+	+	+	+	+	+	+	+	+	[+]	+	+	+	+	+	+	+	+	+
Oxidase, Kovacs'	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ONPG (β-galactosidase)	[+]	+	+	+	+	−	−	−	−	+	+	+	+	d	+	+	−	+	d	+
Yellow pigment	−	−	−	−	−	−	−	−	−	−	[+]	−	−	−	+	+	−	−	−	−
D-Mannose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a]Symbols: +, 90–100% of strains are positive; [+], 76–89% positive; d, 26–75% positive; [−], 11–25% positive; −, 0–10% positive. Data are calculated for a 48-h incubation period unless otherwise indicated (gelatin liquefaction and deoxyribonuclease). The incubation temperature was 36 ± 1°C for all species except *Yersinia ruckeri* and *Xenorhabdus* species, which were incubated at 25 ± 1°C.

Table 5.3—*continued*

Characteristics	*Klebsiella oxytoca*	*Klebsiella pneumoniae* subsp. *ozaenae*	subsp. *pneumoniae*	subsp. *rhinoscleromatis*	*Kluyvera ascorbata*	*Kluyvera cryocrescens*	*Morganella morganii*	*Obesumbacterium proteus* biogroup 1	*Obesumbacterium proteus* biogroup 2	*Proteus mirabilis*	*Proteus myxofaciens*	*Proteus vulgaris*	*Providencia alcalifaciens*	*Providencia rettgeri*	*Providencia stuartii*	*Rahnella aquatilis*	*Salmonella* I	*Salmonella* II	*Salmonella* III = Arizona
Indole production	+	−	−	−	[+]	[+]	+	−	−	−	−	+	+	+	+	−	−	−	−
Methyl red	d	+	[−]	+	+	+	+	+	−	+	+	+	+	+	+	[+]	+	+	+
Voges-Proskauer	+	−	+	−	−	−	−	d	−	[−]	+	−	−	−	−	+	−	−	−
Citrate, Simmons'	+	d	+	−	+	[+]	−	−	−	d	d	[−]	+	+	+	+	+	+	+
Hydrogen sulfide on TSI	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−	−	+	+	+
Urease, Christensen's	+	−	+	−	−	−	+	−	−	+	+	+	−	+	d	−	−	−	−
Phenylalanine deaminase	−	−	−	−	−	−	+	−	−	+	+	+	+	+	+	+	−	−	−
Lysine decarboxylase	+	d	+	−	+	d	−	+	+	−	−	−	−	−	−	−	+	+	+
Arginine dihydrolase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	d	+	[+]
Ornithine decarboxylase	−	−	−	−	+	+	+	−	+	+	−	−	−	−	−	−	+	+	+
Motility	−	−	−	−	+	[+]	+	−	−	+	+	+	+	+	[+]	−	+	+	+
Gelatin liquefaction at 22°C	−	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−
KCN, growth in	+	[+]	+	+	+	[+]	+	−	−	+	+	+	+	+	+	−	−	−	−
Malonate utilization	+	−	+	+	+	[+]	−	−	−	−	−	−	−	−	−	+	−	+	+
D-Glucose, acid production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Glucose, gas production	+	d	+	−	+	+	[+]	−	−	+	+	[+]	[+]	−	−	[+]	+	+	+
Lactose	+	d	+	−	+	+	−	−	−	−	−	−	−	−	−	+	−	−	d
Sucrose	+	[−]	+	[+]	+	[+]	−	−	−	[−]	+	+	[−]	[−]	d	+	−	−	−
D-Mannitol	+	+	+	+	+	+	−	+	−	−	−	−	−	+	[−]	+	+	+	+
Dulcitol	d	−	d	−	[−]	−	−	−	−	−	−	−	−	−	−	[+]	+	+	−
Salicin	+	+	+	+	+	+	−	+	−	−	−	[+]	−	d	−	+	−	−	−
D-Adonitol	+	+	+	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−
myo-Inositol	+	d	+	+	−	−	−	−	−	−	−	−	−	+	+	−	d	[−]	−
D-Sorbitol	+	[+]	+	+	d	d	−	−	−	−	−	−	−	−	−	+	+	+	+
L-Arabinose	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+
Raffinose	+	+	+	[+]	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−
L-Rhamnose	+	d	+	+	+	+	−	−	d	−	−	−	−	d	−	+	+	+	+
Maltose	+	+	+	+	+	+	−	−	d	−	+	+	−	−	−	+	+	+	+
D-Xylose	+	+	+	+	+	+	−	−	d	+	−	+	−	−	−	+	+	+	+
Trehalose	+	+	+	+	+	+	[−]	+	+	+	+	d	−	−	+	+	+	+	+
Cellobiose	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−
α-Methyl-D-glucoside	+	d	[+]	−	+	+	−	−	−	−	+	[+]	−	−	−	−	−	−	−
Esculin hydrolysis	+	[+]	+	d	+	+	−	−	−	−	−	[+]	−	d	−	+	−	−	−
Melibiose	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+
D-Arabitol	+	+	+	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
Mucate	+	[−]	[+]	−	+	[+]	−	−	−	−	−	−	−	−	−	d	+	+	d
Lipase, corn oil	−	−	−	−	−	−	−	−	−	+	+	[+]	−	−	−	−	−	−	−
Deoxyribonuclease at 25°C	−	−	−	−	−	−	−	−	−	−	−	d	−	−	−	−	−	−	−
$NO_3^- \rightarrow NO_2^-$	+	[+]	+	+	+	+	[+]	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase, Kovacs'	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ONPG (β-galactosidase)	+	[+]	+	−	+	+	−	−	−	−	−	−	−	−	−	+	−	d	+
Yellow pigment	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-Mannose	+	+	+	+	+	+	+	+	[+]	−	−	−	+	+	+	+	+	+	+

[a]Symbols: +, 90–100% of strains are positive; [+], 76–89% positive; d, 26–75% positive; [−], 11–25% positive; −, 0–10% positive. Data are calculated for a 48-h incubation period unless otherwise indicated (gelatin liquefaction and deoxyribonuclease). The incubation temperature was 36 ± 1°C for all species except *Yersinia ruckeri* and *Xenorhabdus* species, which were incubated at 25 ± 1°C.

Table 5.3—*continued*

Characteristics	*Salmonella* IV	*Salmonella choleraesuis*	*Salmonella gallinarum*	*Salmonella paratyphi* A	*Salmonella pullorum*	*Salmonella typhi*	*Serratia ficaria*	*Serratia fonticola*	*Serratia liquefaciens*	*Serratia marcescens*	*Serratia odorifera*	*Serratia plymuthica*	*Serratia rubidaea*	*Shigella boydii*	*Shigella dysenteriae*	*Shigella flexneri*	*Shigella sonnei*	*Tatumella ptyseos*	*Yersinia enterocolitica*
Indole production	−	−	−	−	−	−	−	−	−	−	d	−	−	[−]	d	d	−	−	d
Methyl red	+	+	+	+	+	+	[+]	+	[+]	[−]	[+]	+	[−]	+	+	+	+	−	+
Voges-Proskauer	−	−	−	−	−	−	[+]	−	[+]	+	[+]	d	+	−	−	−	−	[−]	−
Citrate, Simmons'	+	[−]	−	−	−	−	+	+	+	+	+	d	+	−	−	−	−	−	−
Hydrogen sulfide on TSI	+	d	+	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−
Urease, Christensen's	−	−	−	−	−	−	−	[−]	−	[−]	−	−	−	−	−	−	−	−	[+]
Phenylalanine deaminase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	[+]	−
Lysine decarboxylase	+	+	+	−	+	+	−	+	+	+	+	−	d	−	−	−	−	−	−
Arginine dihydrolase	d	d	−	[−]	d	−	−	−	−	−	−	−	−	[−]	−	−	−	−	−
Ornithine decarboxylase	+	+	−	+	+	−	−	+	+	+	d	−	−	−	−	−	+	−	+
Motility	+	+	−	+	−	+	+	+	+	+	+	d	[+]	−	−	−	−	−	−
Gelatin liquefaction at 22°C	−	−	−	−	−	−	+	−	+	+	+	d	+	−	−	−	−	−	−
KCN, growth in	+	−	−	−	−	−	d	d	+	+	d	d	[−]	−	−	−	−	−	−
Malonate utilization	−	−	−	−	−	−	−	+	−	−	−	−	[+]	−	−	−	−	−	−
D-Glucose, acid production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Glucose, gas production	+	+	−	+	[+]	−	−	[+]	d	d	−	d	d	−	−	−	−	−	−
Lactose	−	−	−	−	−	−	[−]	+	−	−	+	[+]	+	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−	+	[−]	+	+	d	+	+	−	−	−	−	+	+
D-Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+
Dulcitol	−	−	+	+	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Salicin	d	−	−	−	−	−	+	+	+	+	d	+	+	−	−	−	−	d	d
D-Adonitol	−	−	−	−	−	−	−	+	−	d	d	−	+	−	−	−	−	−	−
myo-Inositol	d	−	−	−	−	−	d	d	d	[+]	+	d	[−]	−	−	−	−	−	d
D-Sorbitol	+	[+]	−	+	[−]	+	+	+	[+]	+	+	d	−	d	d	d	−	−	+
L-Arabinose	+	−	[+]	+	+	−	+	+	+	−	+	+	+	+	d	d	+	−	+
Raffinose	−	−	−	−	−	−	d	+	+	−	d	+	+	−	−	d	−	[−]	−
L-Rhamnose	+	+	−	+	+	−	d	d	[−]	−	+	−	−	−	d	−	[+]	−	−
Maltose	+	+	+	+	−	+	+	+	+	+	+	+	+	[−]	[−]	d	+	−	d
D-Xylose	+	+	d	−	[+]	[+]	+	[+]	+	−	+	+	+	[−]	−	−	−	−	d
Trehalose	+	−	d	+	[+]	+	+	+	+	+	+	+	+	[+]	[+]	d	+	+	+
Cellobiose	[−]	−	−	[−]	−	−	+	−	[−]	[−]	+	[+]	+	−	−	−	−	−	[+]
α-Methyl-D-glucoside	−	−	−	−	−	−	−	+	−	−	−	d	−	−	−	−	−	−	−
Esculin hydrolysis	−	−	−	−	−	−	+	+	+	+	d	[+]	+	−	−	−	−	−	[−]
Melibiose	+	d	−	+	−	+	d	+	[+]	−	+	+	+	[−]	−	d	[−]	d	−
D-Arabitol	−	−	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−	−	d
Mucate	−	−	d	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lipase, corn oil	−	−	−	−	−	−	[+]	−	[+]	+	d	d	+	−	−	−	−	−	d
Deoxyribonuclease at 25°C	−	−	−	−	−	−	+	−	+	+	+	+	+	−	−	−	−	−	−
$NO_3^- \rightarrow NO_2^-$	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Oxidase, Kovacs'	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
ONPG (β-galactosidase)	−	−	−	−	−	−	+	+	+	+	+	[+]	+	−	d	−	[+]	−	+
Yellow pigment	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
D-Mannose	+	[+]	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

[a]Symbols: +, 90–100% of strains are positive; [+], 76–89% positive; d, 26–75% positive; [−], 11–25% positive; −, 0–10% positive. Data are calculated for a 48-h incubation period unless otherwise indicated (gelatin liquefaction and deoxyribonuclease). The incubation temperature was 36 ± 1°C for all species except *Yersinia ruckeri* and *Xenorhabdus* species, which were incubated at 25 ± 1°C.

Table 5.3—*continued*

Characteristics	*Yersinia frederiksenii*	*Yersinia intermedia*	*Yersinia kristensenii*	*Yersinia pestis*	*Yersinia pseudotuberculosis*	*Yersinia ruckeri*	*Xenorhabdus luminescens*	*Xenorhabdus nematophilus*
Indole production	+	+	d	−	−	−	d	d
Methyl red	+	+	+	[+]	+	+	−	−
Voges-Proskauer	−	−	−	−	−	−	−	−
Citrate, Simmons'	[−]	−	−	−	−	−	d	−
Hydrogen sulfide on TSI	−	−	−	−	−	−	−	−
Urease, Christensen's	[+]	[+]	+	−	+	−	[−]	−
Phenylalanine deaminase	−	−	−	−	−	−	−	−
Lysine decarboxylase	−	−	−	−	−	[+]	−	−
Arginine dihydrolase	−	−	−	−	−	−	−	−
Ornithine decarboxylase	[+]	+	+	−	−	+	−	−
Motility	−	−	−	−	−	[+]	+	+
Gelatin liquefaction at 22°C	−	−	−	−	−	d	d	[+]
KCN, growth in	−	−	−	−	−	d	−	−
Malonate utilization	−	−	−	−	−	−	−	−
D-Glucose, acid production	+	+	+	+	+	+	[+]	[+]
D-Glucose, gas production	d	[−]	d	−	−	[−]	−	−
Lactose	d	d	d	−	−	−	−	−
Sucrose	+	+	−	−	−	−	−	−
D-Mannitol	+	+	+	+	+	+	−	−
Dulcitol	−	−	−	−	−	−	−	−
Salicin	[+]	+	d	d	[−]	−	−	−
D-Adonitol	−	−	−	−	−	−	−	−
myo-Inositol	[−]	[−]	d	−	−	−	−	−
D-Sorbitol	+	+	+	−	−	−	−	−
L-Arabinose	+	+	+	+	d	−	−	−
Raffinose	d	d	−	−	[−]	−	−	−
L-Rhamnose	+	+	−	−	+	−	−	−
Maltose	+	+	+	[+]	+	+	[−]	−
D-Xylose	+	+	+	+	+	−	−	−
Trehalose	−	+	+	+	+	+	−	−
Cellobiose	+	+	+	−	−	−	−	−
α-Methyl-D-glucoside	−	[+]	−	−	−	−	−	−
Esculin hydrolysis	[+]	+	−	[+]	+	−	−	−
Melibiose	−	[+]	−	d	+	−	−	−
D-Arabitol	+	d	d	−	−	−	−	−
Mucate	−	−	−	−	−	−	−	−
Lipase, corn oil	d	[−]	−	−	−	d	−	−
Deoxyribonuclease at 25°C	−	−	−	−	−	−	−	[−]
$NO_3^- \rightarrow NO_2^-$	+	+	+	[+]	+	[+]	−	[−]
Oxidase, Kovacs'	−	−	−	−	−	−	−	−
ONPG (β-galactosidase)	+	+	+	[+]	d	d	−	−
Yellow pigment	−	−	−	−	−	−	d	d
D-Mannose	+	+	+	+	+	+	+	[+]

[a]Symbols: +, 90–100% of strains are positive; [+], 76–89% positive; d, 26–75% positive; [−], 11–25% positive; −, 0–10% positive. Data are calculated for a 48-h incubation period unless otherwise indicated (gelatin liquefaction and deoxyribonuclease). The incubation temperature was 36 ± 1°C for all species except *Yersinia ruckeri* and *Xenorhabdus* species, which were incubated at 25 ± 1°C.

type strain for *E. herbicola*, *E. stewartii* and *E. uredovora*; therefore, there is a scientific as well as a nomenclatural problem. For this reason, the *Enterobacteriaceae* Subcommittee, without prejudice, put all four names on the Approved Lists. Three additional names on the Approved Lists, *Escherichia adecarboxylata*, *Erwinia ananas* (now a variety of *Erwinia herbicola*) and *Erwinia milletiae*, may also represent different species now included in this very heterogeneous group. Further studies are required before a final determination can be made with respect to classification and nomenclature. At present, the phytopathologists are comfortable with the three-species concept and many medical bacteriologists use *E. agglomerans*—thus, the use of both systems in this volume.

Hafnia. DNA hybridization studies on *H. alvei* strains revealed two separate relatedness groups (Steigerwalt et al., 1975). A second species

Table 5.4.
Additional biochemical reactions of **Enterobacteriaceae**[a]

Species	Nitrate Reductase	Tetrathionate Reductase	Galacturonate	2-Keto-gluconate	γ-Glutamyl-transferase
Citrobacter amalonaticus (Levinea amalonatica)		+		+	+
Citrobacter diversus (C. koseri, L. malonatica)		−		+	+
Citrobacter freundii	+A	+	+	+	+
Edwardsiella hoshinae				−	
Edwardsiella tarda	+B	+		−	−
Erwinia carotovora	+A	−	+	−	+
Enterobacter aerogenes	+A	−	+	+	+
Enterobacter agglomerans	+A	−	+	+	+
Enterobacter cloacae	+A	−	d	+	+
Enterobacter gergoviae				+	+
Enterobacter sakazakii				+	
Escherichia coli	+A	−		−	+
Hafnia alvei	dA or B	d	+	+	+
Klebsiella oxytoca	dA	d		+	+
Klebsiella ozaenae		−		+	+
Klebsiella pneumoniae	+A	−		+	+
Klebsiella rhinoscleromatis		−		d	−
Morganella morganii	dA	+		−	+
Proteus mirabilis	+A	+		−	+
Proteus vulgaris	+A	+		−	+
Providencia alcalifaciens	+B	+		−	+
Providencia rettgeri	+A	+		d	+
Providencia stuartii	+A	+		−	+
Salmonella subgenus I	+A	+	−	−	+
Salmonella subgenus II	+A	+	+	−	+
Salmonella subgenus III monophasic	+A	+	−	−	−
Salmonella subgenus III diphasic	+A	+	+	−	+
Salmonella subgenus IV	+A	+	+	−	+
Serratia ficaria				+	+
Serratia liquefaciens	+A	+	+	+	+
Serratia marcescens	+A	d	+	+	+
Serratia odorifera	+A	−		+	+
Serratia plymuthica	+A	−	+	+	+
Serratia rubidaea (S. marinorubra)	+A	−	+	+	+
Shigella boydii	+A	−		−	d
Shigella dysenteriae		−		−	d
Shigella flexneri	+A	−		−	d
Shigella sonnei	+A	−		−	−
Yersinia enterocolitica	+B	d		d	+
Yersinia pestis	dB	d		−	−
Yersinia pseudotuberculosis	+B	−		−	+
Yersinia frederiksenii	+B	+			
Yersinia intermedia	+A or B	+			
Yersinia kristensenii	+B	d			

[a] Symbols: +, 90% or more of strains are positive; d, 10.0%–89.9% positive; −, 0–9.9% positive; blank space, data not reported or not available; A, type A; B, type B. The nitrate reductase test was incubated at 32°C; all other tests were incubated at 35–37°C. The γ-glutamyltransferase test was read at 24 h; all other tests results were read at 48 h. Data compiled from the following references: Pichinoty et al. (1969); Le Minor et al. (1979); Richard (1977); Giammanco et al. (1980); Buissière et al. (1981); Grimont et al. (1978); Grimont (1977); Bercovier et al. (1980); Bercovier et al. (1980); Bercovier et al. (1980); Brenner et al. (1980); and Ursing et al. (1980).

was not designated because no single biochemical test or series of tests served to unequivocally separate the two DNA relatedness groups (F.W. Hickman and J.J. Farmer, III, personal communication). *Obesumbacterium proteus* ("*Hafnia protea*") is considered in the section on "Other Genera."

Serratia. *Serratia rubidaea* and *Serratia marinorubra* are subjective synonyms; both appear on the Approved Lists, but have different type strains (the type for *S. marinorubra* is ATCC 27614, not ATCC 27593 as incorrectly shown on the Approved Lists because of an error by the *Enterobacteriaceae* Subcommittee). The *Enterobacteriaceae* Subcommittee must address this problem. *Serratia proteamaculans* was proposed by Grimont and Starr (1978) as a senior synonym for *Serratia liquefaciens*. Holmes (1980) then requested that the epithet *liquefaciens* be conserved over *proteamaculans* because of its worldwide acceptance. This controversy may resolve itself because Grimont et al. (1981) have studied additional strains and have concluded that *S. proteamaculans* is a species distinct from *S. liquefaciens* (see *Serratia* chapter). *Serratia plymuthica* had species status in the seventh edition of *Bergey's Manual* (Breed and Murray, 1957), but was considered a biogroup of *S. marcescens* in the eighth edition (Sakazaki, 1974). DNA relatedness studies have now shown *S. plymuthica* to be a separate species (Grimont et al., 1978).

Proteus, Providencia, *and* Morganella. Biochemical, serologic, guanine plus cytosine content, and DNA relatedness data precipitated several nomenclatural and taxonomic changes (Brenner et al., 1978) in the genus *Proteus* as constituted in the eighth edition of *Bergey's Manual* (Lautrop, 1974). *Proteus morganii* was moved to a new genus, *Morganella*, which on the basis of mol% G + C content and DNA relatedness showed a closer relationship to other genera of *Enterobacteriaceae* than to *Proteus* or *Providencia*. *Proteus inconstans* was moved to the genus *Providencia* as two species, *Providencia alcalifaciens* (*P. inconstans* biogroup A) and *Providencia stuartii* (*P. inconstans* biogroup B). *Proteus rettgeri* was transferred to the genus *Providencia*. The names *Proteus morganii* and *Proteus rettgeri* appear on the Approved Lists. *Proteus myxofaciens*, thought not to be a *Proteus* species in the eighth edition (Lautrop, 1974), has been shown to be a valid and separate species in the genus *Proteus* (Brenner et al., 1978). There is only one available strain of *P. myxofaciens* and, therefore, most people have no familiarity with this species.

Yersinia. Three groups of *Yersinia* previously considered as either biochemically atypical *Y. enterocolitica* or *Y. enterocolitica*-like strains have now been speciated. The new species, *Yersinia intermedia*, *Yersinia frederiksenii* and *Yersinia kristensenii* are separable from *Y. enterocolitica* and from each other by their fermentation reactions for L-rhamnose, raffinose, melibiose and sucrose (Brenner et al., 1980; Ursing et al., 1980; Bercovier et al., 1980). *Y. pestis* and *Y. pseudotuberculosis* were shown to be a single species (Bercovier et al., 1980). It was proposed that they be referred to as *Y. pseudotuberculosis* subsp. *pseudotuberculosis* and *Y. pseudotuberculosis* subsp. *pestis* for taxonic purposes and be written as separate species for medical purposes. This presents a problem because *Y. pestis* is the type species of *Yersinia*. *Yersinia ruckeri*, a fish pathogen, was included in the genus *Yersinia* as an alternative to creating a new genus for this organism (Ewing et al., 1978). "*Yersinia*" *philomiragia* appears on the Approved Lists. This species, first proposed in 1969 (Jensen et al., 1969), was not mentioned in the eighth edition of *Bergey's Manual*. Recent work indicates that it is not a member of *Yersinia* or of the family *Enterobacteriaceae* (Ursing et al., 1980).

Erwinia. The taxonomic problems with *Erwinia herbicola*, *Erwinia stewartii*, *Erwinia uredovora* and *Enterobacter agglomerans* have already been discussed under *Enterobacter*. Also discussed under *Enterobacter* were "*Erwinia*" *dissolvens*, "*Erwinia*" *nimipressuralis* and "*Erwinia*" *cancerogena*. *Erwinia paradisiaca* appears on the Approved Lists. Very little is known about this organism, and strains have not been readily available. All of the so-called soft-rot or Carotovora group appear on the Approved Lists as both *Erwinia* species and as *Pectobacterium* species. Plant pathologists prefer not to split erwiniae at the genus level. The *Enterobacteriaceae* Subcommittee is expected to come to a similar conclusion, and, thus the genus *Pectobacterium* will not be used.

Erwiniae have been mainly studied by phytomicrobiologists and phytopathologists. The media, biochemical and other phenotypic tests used for their isolation, enrichment cultivation and identification are quite different from those used for other *Enterobacteriaceae*. The 37°C incubation temperature used for other *Enterobacteriaceae* is near, at, or above the maximum growth temperatures of erwiniae (excluding the Herbicola group). The isolation of erwiniae from humans or animals is rarely reported. It is not known, however, if they are actually rarely seen or whether their seeming lack of occurrence reflects improper isolation and enrichment procedures, or, if they are isolated, failure to identify them. A study using optimum isolation procedures and an optimum incubation temperature would help resolve this problem. Also needed is a large-scale characterization study of all *Erwinia* species by tests and methods used for other *Enterobacteriaceae*.

DNA relatedness data (Gardner and Kado, 1972; Brenner et al., 1973; Brenner et al., 1974) and phenotypic characteristics indicate the existence of extreme heterogeneity among *Erwinia* species; several species are more closely related to members of *Enterobacter* than to other erwiniae. Starr and Chatterjee (1972), in considering this heterogeneity, reviewed and espoused the possibility of reclassifying erwiniae into one or more existing genera in *Enterobacteriaceae*.

Enterobacteriaceae *and its type genus.* Rule 21a of the *International Code of Nomenclature of Bacteria* requires that family names be formed by adding "aceae" to the stem of the type genus (Lapage et al., 1975). Rahn proposed the name *Enterobacteriaceae* before there was an international code. When the first code was written in 1948, *Enterobacteriaceae* Rahn became illegitimate, not because of its ending, but because it was not in accord with several provisions of the Code. *Enterobacteriaceae* had become so widely used and accepted that in 1958 the Judicial Commission voted to conserve the name and to designate *Escherichia* as the type genus of the family (Judicial Commission, 1958). This Judicial Commission ruling (Opinion 15) was incorporated into the 1958 version of the code and remains in the current 1975 version (Lapage et al., 1975).

In 1978 (International Committee on Systematic Bacteriology, 1979) the Judicial Commission voted to change the family name to "*Enterobacteraceae*" (no "i") and to change the type genus of the family to *Enterobacter*. Several arguments have been raised in opposition to changing the family name. These are based upon the principle of nomenclatural stability, an apparent conflict with the letter and the spirit of several rules in the *International Code of Nomenclature of Bacteria*, and upon the question of whether the Judicial Commission action was procedurally legitimate. These objections have been presented in detail in the *International Journal of Systematic Bacteriology* (Farmer et al., 1980).

A further, more acute problem arose when neither *Enterobacteriaceae* nor "*Enterobacteraceae*" appeared in the body of the Approved Lists (*Enterobacteriaceae* was mentioned in a footnote). Some interpreted this omission to mean that *Enterobacteriaceae* Rahn 1937 as conserved by Judicial Commission Opinion 15 in 1958 had no standing in the literature and that the family was without a name. The name *Enterobacteriaceae* was therefore reproposed (Ewing et al., 1980). The Judicial Commission recently reviewed the present status of the name and concluded that *Enterobacteriaceae* Rahn 1937 is presently valid (Judicial Commission of the International Committee on Systematic Bacteriology, 1981). This dispute will no doubt have to be settled by a decision from the Judicial Commission.

Biochemical Identification of Enterobacteriaceae

Enteric Section, Centers for Disease Control.* People tend to accept without question the reactions given in biochemical charts for various

* Mary Alyce Asbury, Don J. Brenner, Geraldine H. Carter, Betty R. Davis, C. Elais, J.J. Farmer, III, F.W. Hickman, Alma M. McWhorter, Conradine Riddle, and H.G. Wathen. Data compiled and tabulated by J.J. Farmer, III, C. Elais, and W.H. Ewing; section written by Don J. Brenner.

species. To do so, especially in regard to *Enterobacteriaceae*, is a dangerous practice. Results vary according to a number of parameters, some of which are size of inoculum, incubation temperature, duration of incubation, composition and volume of media, test method, criteria for judging a test positive, and the environment from which the tested strains were obtained. Many of these parameters are often not given. Biochemical reactions for *Enterobacteriaceae* are presented in Table 5.3. In an effort to be at least semiquantitative, symbols are given for five (rather than three) percentage ranges. All species were studied in a single laboratory (the Enteric Section at the Centers for Disease Control) by a single set of described methods (Edwards and Ewing, 1972; Hickman and Farmer, 1978). For some species, where the Enteric Section data were biased due to a large number of biochemically atypical strains, the percentages were adjusted to more accurately reflect the percentages expected from a more representative strain sample. Because different methods and tests are often used, the percentage of positive reactions obtained may differ somewhat from those presented in the chapters on specific genera. For example, reactions on *Yersinia* are often done at 28°C. The purpose of Table 5.3 is not to advocate any given test or to put undue emphasis on the percentages obtained, but to present a comprehensive comparison derived from a single set of data obtained by tests commonly done in a diagnostic laboratory. *Erwinia* species and certain of the newly described species are not included because data on these species are insufficient. It must be emphasized that the data in Table 5.3 were obtained at 36 ± 1°C after *48 h of incubation*. For example, *Yersinia enterocolitica* strains are more than 99% positive for urea, but, in our hands, *at 48 h*, slightly less than 90% are positive.

From 50 to more than 200 biochemical tests have been used in phenetic or numerical taxonomic studies of *Enterobacteriaceae* (Bascomb et al., 1971; Johnson et al., 1975; Véron, 1975; Véron and Le Minor, 1975a; Véron and Le Minor, 1975b). These include tests for the fermentation of a large number of carbohydrates and polyhydroxyl alcohols; tests for the ability to use a wide variety of organic substrates as the sole source of carbon and energy, and tests for the presence of specific enzymes. A number of these tests are useful for the differentiation of species or biogroups within *Enterobacteriaceae* (Véron and Le Minor, 1975a; Véron and Le Minor, 1975b). Some tests of particular diagnostic value are for nitrate reductase type A or type B (Pichinoty and Piéchaud, 1968; Pichinoty et al., 1969), tetrathionate reductase (Richard, 1977), fermentation or growth on sodium galacturonate (Le Minor, Buissière and Brault, 1979), presence of α-glutamyltransferase (Giammanco et al., 1980), and fermentation or growth on 2-ketogluconate (Buissiere et al., 1981). A summary of data obtained by the Institut Pasteur group for these tests is given in Table 5.4.

Enterobacteriaceae will soon contain some 20 genera with more than 100 species. To identify the new species it will often be necessary to use tests that are not now used routinely. Furthermore, it is increasingly difficult and risky to identify a strain on the basis of a small number of biochemical characteristics. Each laboratory, depending on its area of specialization, must make several basic decisions. The first is which "nonroutine" tests to add for routine use or for use in special cases. A second decision is whether to speciate in all cases, a third is which species to ignore, and an important corollary of these is what percentage of incorrect identification a laboratory is willing to tolerate. For example, current knowledge indicates that a brewery laboratory must be concerned with *Obesumbacterium*, but probably not *Xenorhabdus*; that fishery laboratories must cope with all *Edwardsiella* species and *Yersinia ruckeri*, but not *Erwinia*; that agricultural laboratories must be aware of *Serratia ficaria* and *Klebsiella*, but not *Edwardsiella*; etc. These are rules of thumb, not absolutes, that allow a laboratory to decrease its work load without significantly decreasing its efficiency.

If there is a certainty with respect to *Entérobactériaceae* it is that the family will continue to be dynamic and will continue to pose a challenge to microbiologists in all specialties.

Acknowledgments

I am extremely grateful to my colleagues in the Enteric Section: Mary Alyce Asbury, Geraldine A. Carter, Betty R. Davis, J. J. Farmer, III, Frances W. Hickman, Alma C. Murlin, Conradine F. Riddle, Arnold G. Steigerwalt and H. Gail Wathen; and to Leon Le Minor, Service des Entérobactéries, Institut Pasteur, Paris, for providing unpublished data. I am indebted to J. J. Farmer, III, and Leon Le Minor for critically reviewing this manuscript. The literature search for this manuscript was concluded on December 30, 1980.

Genus I. **Escherichia** *Castellani and Chalmers 1919, 941*[AL]*

FRITS ØRSKOV

Esch.er.i'chi.a. M.L. fem. n. *Escherichia* named after Theodor Escherich, who isolated the type species of the genus.

Straight rods, 1.1–1.5 μm × 2.0–6.0 μm, occurring singly or in pairs. Capsules or microcapsules occur in many strains. Gram-negative. **Motile by peritrichous flagella or nonmotile.** Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. The remainder of the description is restricted to *E. coli* because *E. blattae* is not well studied and only a few strains exist. Optimum temperature, 37°C. Colonies on nutrient agar may be smooth (S), low convex, moist, gray, with a shiny surface and entire edge and easily dispersible in saline, or they may be rough (R), dry and difficult to disperse well in saline. There are intermediate forms between these extremes. Mucoid and slime-producing forms occur. Chemoorganotrophic. Oxidase-negative. Acetate can usually be used as a sole carbon source, **but citrate cannot be used. Glucose and other carbohydrates are fermented with the production of pyruvate, which is further converted into lactic, acetic and formic acids. Part of the formic acid is split by a complex hydrogenlyase system into equal amounts of CO_2 and H_2.** Some strains are anaerogenic. **Lactose is fermented by most strains but fermentation may be delayed or absent.** Occur in the lower part of the intestine of warm-blooded animals and, in the case of *E. blattae*, of cockroaches. The mol% G + C of the DNA is 48–52 (T_m).

Type species: *Escherichia coli* (Migula 1895) Castellani and Chalmers 1919, 941.

Further Descriptive Information

Many strains, especially those isolated from extraintestinal sites, have polysaccharide capsules or microcapsules (Ørskov et al., 1977).

According to the state of the lipopolysaccharide (LPS) of the outer membrane, strains can be described as smooth (S) or rough (R). S forms, which usually grow as glistening colonies on ordinary agar media and show turbid growth in fluid media, have developed polysaccharide side chains whereas R forms, which usually will show dry and wrinkled colony forms on agar and which will agglutinate spontaneously in fluid media, have lost their polysaccharide side chains by mutation (Lüderitz et al., 1966).

In addition to the proteinaceous flagella, most strains have fimbriae (pili) or fibrillar proteins often extending in great numbers from the bacterial surface and far out into the surrounding medium. They have a width of 5–9 nm (Duguid, 1964; Brinton, 1965). Some fimbriae have specific functions as adhesive organs.

Two main varieties of fimbriae have been described based on their hemagglutinating ability. One is made up of the so-called type 1

* "*AL*" denotes the inclusion of this name on the Approved Lists of Bacterial Names (1980).

fimbriae (pili) characterized by hemagglutination (HA) that is inhibited by mannosides, the HA being mannose-sensitive (MS). Fimbriae of the other variety also cause HA, but the reaction is not inhibited by mannose, the HA being mannose-resistant (MR). Type 1 fimbriae are found in a great majority of *E. coli* strains and constitute antigenically, as far as they have been examined, a group of more or less related antigens (Gillies and Duguid, 1958), while there are many antigenically different MR fimbriae (Ørskov et al., 1980b; Ørskov et al., 1982). It has been shown by Ørskov et al. (1980a) that an important function of type 1 fimbriae is to bind to mucous material (slime) on mucous surfaces. They suggested that the binding of type 1 fimbriae to Tamm-Horsfall protein (urinary slime) is an important part of unspecified host defense. The many serologically diverse MR fimbriae which often function as virulence factors can be both species-specific and organ-specific in their adhesive characters.

Some strains of *E. coli* produce enterotoxins. Two enterotoxins have been well studied: the thermolabile toxin (LT), which is closely related to choleratoxin, and the thermostable toxin (ST). Both are found alone or together in enterotoxigenic *E. coli* (ETEC) strains, and are often associated with a limited number of O:K:H serovars and O groups. ETEC strains often have MR fimbriae.

LT and ST are plasmid-determined. LT can be demonstrated by several techniques. Some are based on LT's ability to stimulate hormone-producing tissue cultures and thereby changing their morphology, others on its immunological properties. ST is demonstrated by the infant mouse assay. For a review, see Rowe (1979).

Subdivision of *E. coli* can be carried out in many ways, but serology is one of the most useful ways to subdivide the species on a global basis. This method is based on the many antigenic differences found in structures on the bacterial surface. The main aspect of this analysis is the O antigen determination based on antigenicity of the LPS; 171 O antigens are presently listed, many of which cross-react.

The K antigens, which originally were defined exclusively according to their agglutinating abilities, have been redefined (Ørskov et al., 1977) and now the definition is also based on their chemical nature. The K antigens are the polysaccharide capsular antigens. Nearly 80 different K antigens are known. A description of the serology, chemistry and genetics of *E. coli* O and K antigens is given by Ørskov et al. (1977).

Flagellar or H antigens make up the third main group of serotyping antigens. A total of 56 H antigens are established. A serovar is recorded in the following way: 018acK1:H7 or 0111:H2 (the latter antigenic formula indicates that K antigens are not present in the strain). MR fimbriae, which are present only in some, often pathogenic, serovars, can also be used for the serological characterization (Ørskov et al., 1977, 1980b). Thus, enterotoxigenic strains from newborn piglets will usually belong to a limited number of serovars and in addition carry fimbrial antigens which are responsible for the necessary adhesion of the strain to the epithelium of the small intestine. The most common antigen, which was originally described as K88 at a time when its chemical character was unknown, is now termed F4 (Ørskov and Ørskov, in Bergan and Norris (eds.): *Methods in Microbiology*, London, Academic Press, in press). Similarly, enterotoxigenic strains isolated from newborn calves may carry an adhesive virulence factor originally named K99, now F5. Since the proteinaceous nature of these MR fimbrial antigens was recognized and more and more similar antigens were found in strains isolated not only from diarrheal diseases but also from extraintestinal diseases, a special new category was proposed for such fimbrial and fibrillar antigens: the F antigens. Thus, K88 is now F4, and K99 is F5. The labels proposed for the CF1 and CF2 antigens found in human enterotoxigenic strains will be F2 and F3, respectively (Ørskov and Ørskov, in Bergan and Norris (eds.): *Methods in Microbiology*, London, Academic Press, in press). Some of the MR fimbriae are plasmid-determined. The MS fimbriae, which for many reasons make up the type 1 group with a separate position from the MR fimbriae, are designated as F1, but this antigen number covers a large group of antigens probably sharing common factors.

From the above description of the many known surface antigens in *E. coli*, it is easy to understand that the number of possible serovars is extremely high and, even though complete serotyping involving O, K and H anigens has been carried out in only a very few laboratories, it is well known that the existing number of serovars is very high.

For a description of other methods for subdivision of *E. coli*, i.e., phage typing, colicin typing, biotyping, typing by outer membrane protein (OMP) pattern, typing by antibiotic resistance patterns and typing by direct hemagglutination, see Ørskov and Ørskov (in Bergan and Norris (eds.): *Methods in Microbiology*, London, Academic Press, in press). Very useful is phage typing of the K1 antigen because K1 antisera are difficult to produce (Gross et al., 1977).

E. coli can be looked upon as primarily an opportunistic pathogen, but investigations in recent years have shown that a rather limited number of serovars or clones also play important and more specific roles in intestinal and extraintestinal diseases. Such clones often possess plasmids which provide them with special virulence traits (Ørskov and Ørskov, 1977).

E. blattae has not been associated with pathogenicity either in humans or in cockroaches.

Extraintestinal diseases. Neonatal meningitis is frequently associated with serovars that have the K1 antigen (Sarff et al., 1975). A limited number of O:K:H serovars, usually with MR fimbriae (F antigens) and often hemolytic, are associated with invasive urinary tract infections (UTI). Other extraintestinal diseases such as urinary tract injections and septicemia may be associated with similar sets of serovars (Ørskov and Ørskov, 1975).

Intestinal diseases. The letters EPEC (enteropathogenic *E. coli*) cover the few serovars associated with infantile diarrhea, mostly occurring in infant institutions. The pathophysiological role of most EPEC serovars has yet to be established. ETEC (enterotoxigenic *E. coli*) consist of a rather limited number of strains which produce enterotoxins (mostly plasmid-determined) causing diarrhea in animals and man. Many ETEC strains carry adhesive F antigens. A high degree of species specificity is characteristic of these clones. The term EIEC (enteroinvasive *E. coli*) covers those serovars that may cause dysentery-like disease. For a recent review, see Rowe (1979).

Enrichment and Isolation Procedures

Many simple agar media can be used for isolation. Media used for selective isolation from feces usually contain substances that partly or completely inhibit growth of bacteria other than *Enterobacteriaceae* (tetrathionate, deoxycholate, bile salts, etc.). The addition of Maranil (dodecylbenzolsulfonate) at a concentration of 0.005% will inhibit swarming of *Proteus* organisms. For details, see Edwards and Ewing (1972) or Kauffmann (1966) or any catalogue from one of the medium-producing companies. At Statens Seruminstitut, Copenhagen, we use a medium developed in the Media Department of this institute: bromothymol blue (BTB) agar.*

Maintenance Procedures

E. coli strains can be kept alive for many years in beef extract agar stabs (tightly closed, e.g. by corks soaked in melted paraffin wax) or on

* Bromothymol blue agar (selective for *Enterobacteriaceae*). Combine the following ingredients: peptone (Orthana Ltd., Copenhagen), 10.0 g; NaCl, 5.0 g; yeast extract (Oxoid), 5.0 g; and distilled water, 1000 ml. The pH is adjusted to 8.0, agar powder is added, and the preparation is autoclaved at 120°C for 20 min. The following components are then added aseptically from sterile stock solutions: Maranil solution (Paste A75 (dodecylbenzolsulfonate), Henkel, West Germany), 1.0 ml; sodium thiosulfate (50% solution), 2.0 ml; bromothymol blue (Riedel de Haen, West Germany; 1.0% solution), 10.0 ml; lactose (33% solution), 27 ml; and glucose (33% solution), 1.2 ml. The pH is adjusted to 7.7–7.8. In order to obtain optimum results, the amount of glucose must be adjusted for every new batch of yeast extract, peptone and agar. This medium is very useful for differentiation of lactose-fermenting colonies based on their color.

Dorset egg medium. Cultures are initially incubated at 37°C followed by storage in the dark at room temperature (20–22°C). After a few weeks or months such cultures often contain many mutational forms such as R forms and acapsular forms; consequently, we prefer to store important cultures in beef broth containing 10% glycerol at −80°C. Screw-capped vials are used for easy access.

Procedures for Testing Special Characters

Kilian and Bülow (1976) have found that a very high percentage of *Escherichia-Shigella* strains, exclusively among the *Enterobacteriaceae*, produce β-glucuronidase (PGUA test). This test therefore holds promise as a screening test for bacteria belonging to this group.

Differentiationof the genus **Escherichia** *from other genera*

See Table 5.3 of the family *Enterobacteriaceae* for characteristics that can be used to differentiate this genus from other genera of the family.

Taxonomic Comments

The identification of *Escherichia* strains seldom causes problems; however, many studies have shown that "*Escherichia* is a genus (or species) made up of phenotypically variable strains" (Farmer and Brenner, 1977). DNA/DNA hybridization studies have been an invaluable tool for solving problems in this field. The genus *Shigella* is closely related to *Escherichia* and only historical reasons make it acceptable that these two genera are not united. Several typical *Escherichia* types, the above mentioned EIEC types, have been found in recent years which have pathogenic traits that are similar to those of *Shigella*. The Sereny test (Sereny, 1967), which demonstrates the capacity to cause keratoconjunctivitis in the guinea pig, typical of *Shigella* strains, is also found in these special *Escherichia* strains. Day et al. (1981) described a tissue culture technique which can be used as a substitute for the Sereny test. Typically, such dysentery-provoking *Escherichia* strains have O antigens that are closely related or identical to *Shigella* O antigens. Brenner et al. (1972) by DNA reassociation studies found high homology between *Shigella* strains and these special *Escherichia* strains. Not unexpectedly, many strains are phenotypically intermediate between *Escherichia* and *Shigella*, but for obvious reasons a special taxonomic status for such strains is not warranted. In the older literature the name Alkalescens-Dispar can be found, but, as stated by Brenner (1978), this group is virtually indistinguishable from *E. coli* strains and is, in fact, a biogroup of *E. coli* that is anaerogenic, lactose-negative (or delayed) and nonmotile.

While most or all characters which classically have been used for definition of the genus *Escherichia* are chromosomally determined, several traits which are not characteristic of *Escherichia* have in recent years been found in otherwise typical *Escherichia* strains. Lautrop et al. (1971) and Layne et al. (1971) described H_2S-positive strains of *Escherichia*; the H_2S character was plasmid-determined. It is not known which selective forces account for the simultaneous isolation of H_2S-positive *Escherichia* strains in different parts of the world.

Other "forbidden" phenotypic traits have similarly been described in *Escherichia*, many of which are undoubtedly plasmid-determined. Ørskov et al. (1961) found many urease-producing strains among typical serovars from piglet diarrhea. Wachsmuth et al. (1979) demonstrated the plasmid-determined nature of a similar urease-positive phenotype in human *E. coli* strains. Citrate-utilizing *E. coli* strains were described by Washington and Timm (1978) and the plasmid background of similar strains was demonstrated by Sato et al. (1978). Carbon dioxide-dependent cultures can be found (Eykyn and Phillips, 1978). *Escherichia blattae* was isolated from the hindgut of healthy cockroaches in England (Burgess et al., 1973) and on Easter Island (Nogrady and Aubert, personal communication). A citrate-positive, malonate-positive biogroup and a biogroup negative in these reactions were described (Burgess et al., 1973).

Further Reading

Burgess, N.R.H., S.N. McDermott and J. Whiting. 1973. Aerobic bacteria occurring in the hind-gut of the cockroach *Blatta orientalis*. J. Hyg. (Lond.) *71*: 1–7.

Edwards, P.R. and W.H. Ewing. 1972. Identification of *Enterobacteriaceae*, 3rd Ed., Burgess Publishing, Minneapolis, Minn.

Ewing, W.H. and W.J. Martin. 1967. The biochemical reactions of the genus *Escherichia*. Monograph, National Communicable Disease Center, Atlanta, Ga.

Kauffmann, F. 1954. *Enterobacteriaceae*, 2nd Ed., Munksgaard, Copenhagen.

Differentiation of the species of the genus **Escherichia**

Characteristics useful in distinguishing the two species of *Escherichia* are given in Table 5.3 of the family *Enterobacteriaceae*.

List of the species of the genus **Escherichia**

1. **Escherichia coli** (Migula 1895) Castellani and Chalmers 1919, 941.[AL] (*Bacillus coli* Migula 1895, 27.)

co'li. Gr. n. *colon* large intestine, colon; M.L. gen. n. *coli* of the colon:

The characteristics are as described for the genus and as listed in Table 5.3 of the family *Enterobacteriaceae*.

Some O groups, O:H and O:K:H serovars from human *E. coli* enter-

Table 5.5.
Some O Groups, O:H and O:K:H serovars from human **Escherichia coli** *enteropathies*

Infantile Diarrhea	Diarrhea in Adults and Children	
EPEC[a]	ETEC[b]	EIEC[c]
026, 044, 055, 086, 0111, 0114, 0119, 0125, 0126, 0127, 0128, 0142, 0158	O6:K15:H16, O8:K40:H9, O8:K47:H-, O8:K25:H9, O11:H27, O15:H11, O20:H-, O25:K7:H42, O25:K98:H-; O27:H7, O27:H20, O63:H12, O73:H45, O85:H7; O78:H11, O78:H12, O114:H21; O115:[H51],[d] O128:H7, O128:H12, O128:H21, O139:H28, O148:H28, O149:H4, O159:H4, O159:H20, O159:H34; O166:H27, O169:H-	028ac, 0112, 0124, 0136, 0143, 0144, 0152, 0164

[a] EPEC, enteropathogenic *E. coli*. The O groups are listed; however, only a limited number of O:H types have been shown to have an association with infantile diarrhea.
[b] ETEC, enterotoxigenic *E. coli*. The data presented are primarily from Ørskov and Ørskov (1980). 0166 = OX8, and 0169 = OX2.
[c] EIEC, enteroinvasive *E. coli*.
[d] [ ], nonmotile variants exist.

opathies are indicated in Table 5.5 in this chapter.

Occurs in the lower part of the intestine of warm-blooded animals.

The mol% G + C of the DNA is 48–52 (T_m).

Type strain: ATCC 11775.

2. **Escherichia blattae** Burgess, McDermott and Whiting 1973, 4.[AL]

blat'tae. L. fem. n. *blatta* cockroach; L. gen. n. *blattae* of the cockroach.

The characteristics are as described for the genus and as listed in Table 5.3 of the family *Enterobacteriaceae*.

Isolated from the hindgut of the cockroach *Blatta orientalis*.

Type strain: CDC 9005-74.

Species Incertae Sedis

Escherichia adecarboxylata Leclerc 1962, 736.[AL]

a.de.car.box'y.la.ta. Gr. pref. *a* not; M.L. adj. *adecarboxylata* not decarboxylating.

Little additional information concerning this organism has come forward since its mention in the eighth edition of the *Manual*, but unpublished studies indicate that it probably belongs to the *Erwinia herbicola-Enterobacter agglomerans* complex (Bascomb et al., 1971).

Type strain: ATCC 23216.

Genus II. **Shigella** *Castellani and Chalmers 1919, 936* [AL]

BERNARD ROWE AND ROGER J. GROSS

Shi.gel'la. M.L. dim. ending *-ella*; M.L. fem. n. *Shigella* named after K. Shiga, the Japanese bacteriologist who first discovered the dysentery bacillus.

Straight rods similar in morphology to other *Enterobacteriaceae*. Gram-negative. **Nonmotile**. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Catalase-positive (with exceptions in one species). Oxidase-negative. Chemoorganotrophic. **Ferment sugars without gas production** (a few exceptions produce gas). **Do not use citrate or malonate as a sole carbon source. Do not grow in KCN or produce H_2S.** Intestinal pathogens of man and other primates, causing **bacillary dysentery**. The mol% G + C of the DNA is 49–53 (Normore, 1973).

Type species: Shigella dysenteriae (Shiga 1898) Castellani and Chalmers 1919, 935.

Further Descriptive Information

The genus consists of four species, *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*. These are often referred to as subgroups A, B, C and D, respectively.

The biochemical characteristics of the genus are listed in Table 5.6.

The species have been well characterized antigenically. *S. dysenteriae* contains 10 serovars, each with a distinctive antigen by which it can be recognized; there are few cross-reactions, either within the species or with other species.

S. flexneri contains eight serovars and nine subserovars. The serovars are antigenically related, but each has a qualitatively distinct major (type) antigen; the group antigens are shared by other members of the species. Because of the important intragroup relations, highly absorbed sera are needed for the detailed serotyping of *S. flexneri*. The immunochemical and genetic basis of the complex antigenic structure of the species has been summarized by Petrovskaya and Bondarenko (1977). The lipopolysaccharide O antigen of all serovars except *S. flexneri* 6 contains group antigens 3, 4 as a main primary structure. The type-specific antigens I, II, IV and V and the group antigens 7, 8 are all the result of phage conversion of the 3, 4 antigens resulting in the incorporation of α-glycosyl secondary side chains. Type-specific antigen III and group antigen 6 differ from the above antigens in that they contain acetyl groups. Nevertheless, these antigens are also formed as a result of phage conversion of the 3, 4 antigens. The lipopolysaccharide O antigen of *S. flexneri* serovar 6 differs from that of other *S. flexneri* serovars and does not contain the immunochemical determinants of the 3, 4 antigens. Strains of serovar 6 therefore resemble strains of *S. boydii* immunochemically, and Petrovskaya and Bondarenko have proposed that they be reclassified as such.

S. boydii contains 15 serovars and each has a qualitatively distinct antigen; there may be some cross-reactions with antisera to other *Shigella* species, but these seldom interfere with diagnosis. Serovars 10 and 11 share a major antigen, although each possesses a specific antigen.

S. sonnei contains only one serovar, which exists in two "phases," I and II; each has a distinctive antigen. Phase II is regarded as a loss variation, but organisms in that phase may be isolated from patients, usually during convalescence and toward the end of an outbreak. An antiserum containing agglutinins for both phases should be used for identification

In addition to the recognized serovars of shigellae, Ewing et al. (1958) have described a number of provisional *Shigella* serovars. These may be added to the serotyping scheme in the future, but in the meantime they remain *sub judice* and antisera for their identification is usually available only at reference laboratories. Provisional serovars under consideration at present include *S. dysenteriae* 3873-50, 2000-53 and 3341-55 and *S. boydii* 3615-53, 2710-54 and 1621-54.

Colicin typing is of value in epidemiological studies of *S. sonnei*. The scheme is based on that described by Abbott and Shannon (1958) and distinguishes 14 types using 15 indicator strains (see *Procedures for Testing Special Characters*). Phage-typing schemes have also been described. Only a few reports have appeared for *S. dysenteriae* and *S. boydii* but a number of schemes have been described for *S. flexneri* and *S. sonnei* (Bergan, 1979).

Shigellae are pathogens of man and other primates and although there have been occasional reports of infections in dogs, other animals are resistant to infection. Laboratory animals such as mice, rabbits and guinea pigs may be infected orally but only following starvation and treatment with gastric antacids and antiperistaltic agents.

In humans, the lesions of bacillary dysentery are usually restricted to the rectum and large intestine, but in severe cases part of the terminal ileum may be affected. Typically there is acute inflammation with ulceration of the epithelium; the organisms rarely spread deeper than the lamina propria, and bloodstream involvement is uncommon. Infections due to *S. sonnei* rarely extend beyond the epithelial inflammatory stage, but infections with *S. dysenteriae* serovar 1 (Shiga's bacillus) or *S. flexneri* strains often cause ulceration.

The invasive properties of *Shigella* have been demonstrated using tests for the ability to produce keratoconjunctivitis in the guinea pig eye (Sérèny test), and to invade HeLa cells in tissue culture (Ogawa et al., 1967; Day et al., 1981). The rabbit ileal loop test has also been used as an experimental model. It has been shown that *S. dysenteriae* serovar 1 and *S. flexneri* serovar 2a produce toxins which are lethal to mice, enterotoxic in rabbit ileal loops, and cytotoxic for Hela cells (O'Brien et al., 1977). The demonstration of related toxins from both *S. dysenteriae* serovar 1 and *S. flexneri* might suggest that the enterotoxin has a role in the pathogenesis of bacillary dysentery. It was first thought that the enterotoxin of *S. dysenteriae* serovar 1 did not stimulate adenyl cyclase, unlike the cholera enterotoxin and the heat-labile enterotoxin of *Escherichia coli*. However, it has now been shown that under optimum assay conditions adenyl cyclase is stimulated by *S. dysenteriae* serovar 1 enterotoxin (Charney et al., 1976). Further work is needed, and in any case there is little doubt that epithelial invasion and multiplication are the main virulence factors.

Table 5.6.
Characteristics of the genus **Shigella**[a]

Test or Substrate	Result
β-Galactosidase	D[b]
Simmons' citrate	–
Christensen's citrate	–
Sodium acetate	D[c]
Arginine decarboxylase	–
Lysine decarboxylase	–
Ornithine decarboxylase	D[d]
Gelatin liquefaction	–
Gluconate	–
H_2S (triple sugar iron agar)	–
Indole production	D[e]
KCN, growth in	–
Malonate utilization	–
Methyl red test	+
Voges-Proskauer test	–
Phenylalanine deaminase	–
Urease	–
Motility	–
Glucose:	
Acid	+
Gas	D[f]
Acid from:	
Adonitol	–
Cellobiose	–
Dulcitol	–
Inositol	–
Lactose	D[g]
Mannitol	D[h]
Raffinose	D
Salicin	–
Sucrose	D[i]
Xylose	–

[a] Symbols: see standard definitions.
[b] Strains of *S. dysenteriae* 1 and *S. sonnei* are positive; positive strains of *S. flexneri* 2a and *S. boydii* 9 have been described.
[c] Some biovars of *S. flexneri* 4a are positive; all other biovars are negative.
[d] Strains of *S. boydii* 13 and *S. sonnei* are positive.
[e] Some strains of some serovars of *S. dysenteriae*, *S. flexneri* and *S. boydii* produce indole while strains of other serovars are always negative. *S. sonnei* is always negative.
[f] Some biovars of *S. flexneri* 6 are positive; positive strains of *S. boydii* 13 and 14 have been described.
[g] Strains of *S. sonnei* are usually positive after several days of incubation; positive strains of *S. flexneri* 2a and *S. boydii* 9 have been described.
[h] Strains of *S. dysenteriae* are negative; negative biovars of *S. flexneri* 4a ("*S. rabaulensis*," "*S. rio*") and *S. flexneri* 6 (Newcastle biovar) occur; negative biovars of *S. sonnei* occur rarely.
[i] Strains of *S. sonnei* are usually positive after several days of incubation.

Although infections are frequently mild and self-limiting, antibiotic treatment may be required in severe cases. Treatment is complicated by the increasing incidence of multiple drug resistance among *Shigella* strains. Indeed, the first observation of multiple, transferable drug resistance was in *Shigella* in Japan (Ochiai et al., 1959). Subsequent surveys in the United States (Neu et al., 1975) and in England (Thomas and Tillett, 1973) showed that the majority of *S. sonnei* strains were multiply resistant. Furthermore, a recent survey in England and Wales (Gross et al., 1981) showed that almost 50% of *Shigella* strains belonging to subgroups A, B and C were resistant to three or more drugs.

Enrichment and Isolation Procedures

Food and water. The minimum infecting dose of shigellae is small and occurrence of the organisms in food, milk and water may be significant even when only a small number of organisms are present. There are no reliable and effective enrichment methods, however, and the true incidence of *Shigella* contamination of foodstuffs cannot be accurately determined. The GN (Gram-negative) broth of Hajna (1955) may be useful for enrichment of *Shigella* and it is recommended that the investigation of foodstuffs should include an enrichment step using this medium. Subsequent steps in the isolation of *Shigella* from foods should follow the procedure recommended for fecal specimens.

Fecal specimens. Freshly passed stools should be examined, although if this is not possible fecal swabs showing marked fecal staining may be used. The specimens should be collected during the acute stage of the disease and before any chemotherapy is started. Specimens should be examined as soon after collection as possible. Enrichment with GN broth may be of value, but isolation is usually effected by direct plating. If the specimen includes blood and mucus, these should be included in the portion examined.

Some strains grow poorly on inhibitory media, and both a relatively noninhibitory medium such as MacConkey or eosin methylene blue (EMB) agar, and an inhibitory medium such as deoxycholate citrate agar (DCA) or shigella-salmonella (SS) agar should be used. Instructions for preparation of these media are given by Edwards and Ewing (1972). Specimens are streaked onto the chosen media and after overnight incubation at 37°C non-lactose-fermenting colonies are selected for further examination. Even when stool specimens from acute dysentery are examined, there may be only a scanty growth of *Shigella*.

Maintenance Procedures

Cultures of *Shigella* may be maintained on Dorset egg medium at room temperature, but rough and degraded variants frequently arise. Important cultures are best maintained lyophilized or in liquid nitrogen.

Procedures for Testing Special Characters

For colicin typing of *S. sonnei*, the organism under investigation is inoculated heavily in a broad streak across a blood agar plate and incubated at 37°C for 24 h. The bacterial growth is then removed from the agar by scraping with a glass slide and the organisms remaining are killed with chloroform. The 15 indicator strains are streaked onto

Table 5.7.
Differential characteristics of the species of the genus **Shigella**[a]

	S. dysenteriae	*S. flexneri*	*S. boydii*	*S. sonnei*
β-Galactosidase	D[b]	–	D	+
Ornithine decarboxylase	–	–	–[c]	+
Gas from glucose[d]	–	–	–	–
Acid from:				
Dulcitol[e]	–	–	–	–
Lactose	–	–	–	(+)[f]
Mannitol	–	+	+	+
Raffinose	–	D	–	(+)[f]
Sucrose	–	–	–	(+)[f]
Xylose	–	–	D	–
Indole production[g]	D	D	D	–

[a] For symbols see standard definitions.
[b] *S. dysenteriae* 1 strains are positive; some other serovars are sometimes positive.
[c] *S. boydii* 13 strains are positive.
[d] Gas production from glucose: only certain biovars of *S. flexneri* 6, and of *S. boydii* 13 (Rowe et al., 1975) and *S. boydii* 14 (Carpenter, 1961) are aerogenic.
[e] *S. dysenteriae* 5 and *S. flexneri* 6 may ferment dulcitol.
[f] (+), positive reaction delayed (more than 24 h).
[g] *S. dysenteriae* 1, *S. flexneri* 6 and *S. sonnei* never produce indole, while strains of *S. dysenteriae* 2 always produce indole.

the plate at right angles to the original line of growth. After further incubation for 8–12 h the patterns of inhibition of growth of the indicator strains can be examined and compared with a key. It is important that controls be included in every batch of tests.

Differentiation of the genus **Shigella** *from other closely related taxa*

The biochemical identification of *Shigella* is complicated by the similarity of some strains of other genera. In particular, strains of *Hafnia alvei*, *Providencia sp.*, *Aeromonas sp.* and atypical *Escherichia coli* frequently cause difficulties.

Nonlactose-fermenting or anaerogenic strains of *E. coli* are a common problem. Of particular interest are members of the Alkalescens Dispar (A-D) group which are now defined as nonmotile, anaerogenic biotypes of *E. coli*. These are best differentiated from *Shigella* by means of the Christensen's citrate and lysine decarboxylase tests in which *Shigella* is always negative. The members of the A-D group were divided into eight serogroups on the basis of their O antigens (Frantzen, 1950), although most of these are identical with or closely related to *E. coli* antigens. Now that these organisms are regarded as *E. coli*, no further serogroups will be added to the A-D scheme.

Taxonomic Comments

The occurrence of biochemically atypical strains of *E. coli* has prompted Shmilovitz et al. (1974) to suggest the recognition of an intermediate group to be known as Intermediate Shigella Coli Alkalescens Dispar (ISCAD). Stenzel (1978) proposed the inclusion of such

Table 5.8.
Earlier designations and antigenic formulae of **Shigella** *species*

Subgroup and Species	Serovar	Subserovar	Antigenic Formula	Main Earlier Designations or Synonyms
Subgroup A				
S. dysenteriae	1			*S. shigae*
	2			*S. schmitzii*, *S. ambigua*
	3			*S. largei* Q771, *S. arabinotarda* A
	4			*S. largei* Q1167, *S. arabinotarda* B
	5			*S. largei* Q1030
	6			*S. largei* Q454
	7			*S. largei* Q902
	8			Serotype 599-52 (Ewing et al.)
	9			Serotype 58 (Cox and Wallace)
	10			Serotype 2050 (Ewing)
Subgroup B				
S. flexneri	1	1a	I:2,4	V (Andrewes and Inman)
		1b	I:'S':6:2,4	VZ (Andrewes and Inman)
	2	2a	II:3,4	W (Andrewes and Inman)
		2b	II:7,8	WX (Andrewes and Inman)
	3	3a	III:6,7,8	Z (Andrewes and Inman)
		3b	III:6,3,4	
		3c	III:6:	
	4	4a[a]	IV:'B':3,4	103 (Boyd)
		4b	(IV):'B':6:3,4	103Z (Rewell and Bridges)
	5		V:7,8	P119 and P119X (Boyd), (Bridges)
	6		VI:(2),4	*S. newcastle*; Manchester bacillus; Boyd 88 (Newcastle and Manchester-aerogenic; Newcastle-mannitol-negative)
	X		–:7,8	X (Andrewes and Inman)
	Y		–:3,4	Y (Andrewes and Inman)
Subgroup C				
S. boydii	1			170 (Boyd)
	2			P288 (Boyd)
	3			D1 (Boyd)
	4			P274 (Boyd)
	5			P143 (Boyd)
	6			D19 (Boyd)
	7			Lavington I; *S. etousae*
	8			Serotype 112 (Cox and Wallace)
	9			Serotype 1296/7 and 1320 (Francis)
	10			Serotype 430 (Ewing); D15 (Szturm et al.)
	11			Serotype 34 and 732 (Ewing)
	12			Serotype 123 (Ewing and Hucks)
	13			Serotype 425 (Ewing and Hucks)
	14			Serotype 2770-51 (Ewing and Hucks)
	15			Serotype 703 (Ewing et al.)
Subgroup D				
S. sonnei				Duval's bacillus; *B. ceylanesis* A

[a] The group phase of this subserovar, corresponding to Boyd's 103B organism, has the formula –:'B':3,4.

strains in *Shigella* subgroup D and suggested that this subgroup should be renamed "*S. metadysenteriae.*" The situation is further complicated by the fact that some strains of *E. coli* share with *Shigella* the ability to cause bacillary dysentery and to cause keratoconjunctivitis of the guinea pig eye in the Sérèny test (Sakazaki et al., 1974). However, the Enterobacteriaceae Sub-Committee of the International Committee on Bacteriological Nomenclature (Carpenter, 1963) has advised that pathogenicity should not be considered in the classification of *Enterobacteriaceae* and strains with biochemical reactions which do not conform strictly to those of *Shigella* should be classified as atypical *E. coli.* Nevertheless, it should be realized that *E. coli* and *Shigella* strains (except *S. boydii* serovar 13) are indistinguishable on the basis of DNA hybridization studies (Brenner et al., 1973) and it may be largely for historical reasons that the two genera remain separate.

Further Readings

Edwards, P.R. and W.H. Ewing. 1972. Identification of Enterobacteriaceae, Burgess Publishing, Minneapolis, Minn.
Keusch, G.T. 1979. In Lambert (Editor), Clinics in Gastroenterology, Vol. 8, No. 3, W. B. Saunders, London, pp. 645–662.
DuPont, H.L. and L.K. Pickering (Editors). 1980. Infections of the Gastrointestinal Tract, Plenum Medical Book, New York, pp. 61–82.
Lapage, S.P., Rowe, B., Holmes, B. and R.J. Gross. 1979. In Skinner and Lovelock (Editors), Identification Methods for Microbiologists, 2nd Ed., Academic Press, London, pp. 123–141.

Differentiation of the species of the genus **Shigella**

Biochemical characteristics useful for differentiating the species of *Shigella* are listed in Table 5.7.

List of the species of the genus **Shigella**

1. **Shigella dysenteriae** (Shiga 1898) Castellani and Chalmers 1919, 935, epit. spec. cons. Opin. 11, Jud. Comm. 1954, 149.[AL] (*Bacillus dysenteriae* Shiga 1898, 817.)

dys.en.te'ri.ae. Gr. n. *dysenteria* dysentery; M.L. gen. n. *dysenteriae* of dysentery.

Also known as subgroup A.

Colonies of serovar 1 often have a pinkish tinge on Leifson's deoxycholate citrate agar. Catalase is not produced by serovar 1, but is usually produced by strains of other serovars.

Mannitol is not fermented. Dulcitol is fermented by strains of serovar 5. Indole is not produced by serovar 1 but is always produced by strains of serovar 2; strains of other serovars vary in indole production.

All the serovars have, at one time or another, been known by other designations, and these are shown in Table 5.8.

Type strain: ATCC 13313 (NCTC 4837; Newcastle 1934) (Jud. Comm. 1963, Opin. 26).

2. **Shigella flexneri** Castellani and Chalmers 1919, 937, epit. spec. cons. Opin. 11, Jud. Comm. 1954, 149.[AL]

flex'ner.i. M.L. gen. n. *flexneri* of Flexner; named after Simon Flexner, an American bacteriologist.

Also known as subgroup B.

Catalase is produced.

Mannitol is fermented, except by biovar Newcastle, serovar 6 and a mannitol-negative, xylose-positive biovar of serovar 4a (sometimes known as "*S. rabaulensis*"). Dulcitol is fermented by certain biovars of serovar 6 (see Table 5.7), some of which produce gas from fermentable sugars.

Indole is not produced by serovar 6; in other serovars indole production is variable.

The reactions of *S. flexneri* strains in diagnostic absorbed antisera are shown in Table 5.9.

Type strain: ATCC 29903.

3. **Shigella boydii** Ewing 1949, 634, epit. spec. cons. Opin. 11, Jud. Comm. 1954, 149.[AL]

boy'di.i. M.L. gen. n. *boydii* of Boyd; named after Sir John Boyd, a British bacteriologist.

Also known as subgroup C.

Catalase is produced.

Mannitol is fermented. Dulcitol is usually fermented by serovars 2, 3, 4, 6 and 10, but this may be delayed. Xylose fermentation is variable.

Indole may or may not be produced. Gas-producing biovars of *S. boydii* serovar 13 (Rowe et al., 1975) and serovar 14 (Carpenter, 1961) have been described.

Type strain: ATCC 8700.

4. **Shigella sonnei** (Levine 1920) Weldin 1927, 182, epit. spec. cons. Opin. 11, Jud. Comm. 1954, 149.[AL] (*Bacterium sonnei* Levine 1920, 31.)

son'ne.i. M.L. gen. n. *sonnei* of Sonne; named after Carl Sonne.

Also known as subgroup D.

On deoxycholate citrate agar colonies are at first colorless, but after

Table 5.9.
Reactions of **S. flexneri** *serovars in diagnostic absorbed slide-agglutinating serums*[a]

Serum		Serovar with Simplified Antigenic Formula[b]													
Type	Agglutinins	1a I:2,4	1b I:S:6:2,4	2a II:3,4	2b II:7,8	3a[c] III:6:7,8	3b III:6:3,4	3c III:6...	4a IV:B:3,4	4b IV:B:6:3,4	5[d] V:7,8	5 V:(3,4)	6 VI:2,4	X −:7,8	Y −:3,4
1	I	++	++	−	−	−	−	−	−	−	−	−	−	−	−
2	II	−	−	++	++	−	−	−	−	−	−	−	−	−	−
3	III:6	−	+	−	−	++	++	++	−	+	−	−	−	−	−
4	IV:B	−	−	−	−	−	−	−	++	++	−	−	−	−	−
5	V	−	−	−	−	−	−	−	−	−	++	++	−	−	−
6	VI	−	−	−	−	−	−	−	−	−	−	−	++	−	−
X	7,8	−	−	−	++	++	−	−	−	−	+	−	−	++	−
Y	3,4	−/±	−/±	−/+	−	−	++	−	−/+	−/±	−	−/±	−	−	++

[a] Symbols: ++, strong reaction; +, moderate reaction; ±, weak reaction; −, no reaction.
[b] Arabic numerals are used to designate serovars, but it is customary to use Roman numerals to express **type-specific** antigens or agglutinins, and arabic numerals for **group antigens or agglutinins.**
[c] Occasional variants may also react in absorbed Y serum.
[d] Subserovars of *S. flexneri* 5 have not yet been designated.

a few days show bright pink papillae consisting of lactose-fermenting cells. On MacConkey's taurocholate lactose agar, phase I colonies are indistinguishable from colonies of other shigellas, but phase II colonies are larger, flatter and more translucent and have an irregular edge. On subculture, phase I colonies produce both phase I and phase II colonies, but phase II colonies give rise to phase II colonies only.

Mannitol is fermented rapidly, lactose and sucrose more slowly. Some strains may ferment xylose.

Catalase is produced. Indole is not produced.

Ornithine is decarboxylated; arginine may be decarboxylated.

Type strain: ATCC 29930.

Genus III. **Salmonella** *Lignières 1900, 389*[AL]

L. LE MINOR

Sal.mon.el'la. M.L. dim. ending *-ella*; M.L. fem. n. *Salmonella* named after D. E. Salmon, an American bacteriologist.

Straight rods, 0.7–1.5 × 2.0–5.0 μm, conforming to the general definition of the family *Enterobacteriaceae*. Gram-negative. **Usually motile** (peritrichous flagella). Facultatively anaerobic. Colonies are generally 2–4 mm in diameter. Nitrates are reduced to nitrites. **Gas is usually produced from glucose.** Hydrogen sulfide is usually produced on triple-sugar iron agar. Indole-negative. **Citrate is usually utilized as a sole carbon source.** Lysine and ornithine decarboxylase (Møller's) reactions are usually positive. Urease-negative. Phenylalanine and tryptophan are not oxidatively deaminated. Sucrose, salicin, inositol and amygdalin are usually not fermented. Lipase and deoxyribonuclease are not produced. Pathogenic for humans, causing enteric fevers, gastroenteritis and septicemia; may also infect many animal species besides humans. Some serovars are strictly host-adapted. The mol% G + C of the DNA is 50–53 (Ch, T_m, Bd) (Hill, 1966).

Type species: *Salmonella choleraesuis* (Smith 1894) Weldin 1927, 155.

Further Descriptive Information

Although most salmonellae are motile, nonmotile mutants may occur, and one type ("*S. gallinarum*" or "*S. pullorum*") is always nonmotile.

Certain *Salmonella* types may form unusually small colonies (~1 mm diameter), whereas most types form larger colonies (2–4 mm).

Most salmonellae are aerogenic; however, *S. typhi*, an important exception, never produces gas. Anaerogenic variants of normally gas-producing *Salmonella* serovars may occur; this is particularly common with *S. dublin*.

Hydrogen sulfide is produced by most salmonellae, but a few types do not form it (e.g., some strains of *S. choleraesuis*, and most strains of "*S. paratyphi A*.")

Citrate is generally utilized by salmonellae, but some types do not use it (particularly *S. typhi* and "*S. paratyphi-A*"). Most salmonellae do not utilize malonate, but *S. arizonae* does use it.

The lysine decarboxylase reaction (Møller's) is positive for most salmonellae; an important exception is "*S. paratyphi-A*." Most salmonellae are also positive for ornithine decarboxylase (Møller's), but *S. typhi* is negative.

Lactose is generally not fermented by salmonellae, but many strains of *S. arizonae* ferment it rapidly or slowly, and nearly all strains of *S. arizonae* have β-galactosidase activity (by the ONPG test).

Other biochemical characteristics of the genus are indicated in Table 5.3 in the article on the family *Enterobacteriaceae*. Subdivision of the genus *Salmonella* into the so-called "subgenera" of Kauffman (1960, 1963a, b, 1964) on the basis of biochemical characteristics is shown in Table 5.10 of the present chapter. These subdivisions correspond more closely to species or subspecies in other groups of bacteria, but whatever rank is assigned to them, the worthiness of these subdivisions was confirmed by Rohde (1965, 1966, 1967). A new "subgenus," V, is added in the present chapter. Salmonellae belonging to this "subgenus" grow in the presence of KCN (as those of "subgenus" IV); they are lactose-, malonate- and gelatin-negative and dulcitol- and mucate-positive (as those of "subgenus" I); and they are negative for *d*-, *l*- and *i*-tartrate and positive for the ONPG test (as those of "subgenus" III).

Division into serovars. The Kauffmann-White scheme, in which

Table 5.10.
Differential characteristics of the "subgenera" of the genus **Salmonella**[a]

	"Subgenus"				
	I	II	III	IV	V[b]
β-galactosidase (ONPG test)	−	− or x	+	−	+
Acid production from:					
Lactose	−	−	+ or x	−	−
Dulcitol	+	+	−	−	+
Mucate	+	+	d	−	+
Galacturonate[c]	−	+	d	+	+
Utilization of:					
Malonate	−	+	+	−	−
d-Tartrate	+	− or x	− or x	− or x	−
Gelatin hydrolysis (film method)	−	+	+	+	−
Growth in presence of KCN	−	−	−	+	+
Habitat of the majority of strains:					
Warm-blooded animals	+	−	−	−	−
Cold-blooded animals and environment	−	+	+	+	+

[a] Symbols: +, positive for 90% or more of strains in 1–2 days; d, positive for 11–89% of strains in 1–2 days; −, positive for 0–10% of strains in 1–2 days; x, late and irregularly positive (3–7 days). The temperature for all reactions is 37°C.

[b] L. Le Minor, M. Véron and M. Popoff, 1982, Ann. Microbiol. (Inst. Pasteur): *133B*: 223–243.

[c] From Le Minor et al. (1979). Monophasic serovars of "Subgenus" III are galacturonate-negative; diphasic serovars are positive.

organisms are represented by the numbers and letters given to the different O (somatic), Vi (capsular) and H (flagellar) antigens, indicates only those antigens of primary diagnostic importance and is not a complete record of the antigenic complement or its complexity (Kauffmann, 1966). The scheme, expanded to include all five "subgenera," is given in Table 5.11. The original "*Arizona*" antigens (given in brackets) have been converted to the presently used *Salmonella* designations.

Antigenic formulae (for example, 6,7:r:1,7) represent the O antigens: the phase 1 H antigen(s): the phase 2 H antigen(s), respectively. Those formulae with particular O antigens in common are collected into an O group and arranged alphabetically by H antigens within the group.

Lysogenization by certain converting phages may produce changes in the O antigenic formulae of salmonellae. In antigenic groups A, B and D the presence of O antigen 1 (factor 1) is associated with lysogenization (Iseki and Kashiwagi, 1955, 1957; Stocker, 1958; Zinder, 1957), but the presence or absence of this factor in strains of these groups does not change the name of the organism (for example, the name *S. typhimurium* applies to both the "1^{+}" and "1^{-}" strains). On the other hand, in group E the name is changed: phage ϵ_{15} (Iseki and Sakai, 1953) alters the O antigen 3, 10 to 3, *15*, thereby making "*S. anatum*" become "*S. newington*," and in a similar way phage ϵ_{34} changes "*S. newington*" to "*S. minneapolis*." The same applies to "*S. cerro*" and "*S. siegburg*," the latter being merely the lysogenic variant of the former (Le Minor, 1965), and also to all of the strains of group C_4 (O antigen 6,7,14) which are lysogenic variants of group C_1 (0 antigen 6,7) although they bear different names. For this reason, all the factors associated with phage conversion are underlined in the joint Kauffmann-White scheme (Table 5.11) which includes all "*Arizona*" serovars with their corresponding *Salmonella* formulae. The converting phages of *Salmonella* are identical in morphology (Vieu et al., 1965), but their action is limited to certain O groups and they are serologically different from one another (Le Minor, 1968).

The specificities of the O factors in *Salmonella* is determined by the composition and structure of the polysaccharides. Specificity is modified during S → R mutation and by bacteriophage conversions (see reviews by Stocker and Mäkelä, 1971, 1978; Lüderitz et al., 1971). Thus, the only difference between the 4,12 and the 9,12 O-specific repeating units is in the di-deoxyhexose branch unit attached to the mannose, which is abequose in 4,12 and tyvelose in 9,12. In the conversion of 3,10 → 3,15, the terminal acetyl radical of the chain is suppressed and the α-linkage between galactose and mannose is transformed into a β-linkage.

Other modifications of the specificity of somatic (O) antigens may occur after a mutation, resulting in new specificities called T_1 and T_2 by Kauffmann (1956) and in different R types (reviewed by Stocker and Mäkelä, 1971, 1978, and by Lüderitz et al., 1971).

Subdivision of serovars. Biovars are different sugar fermentation patterns shown by strains of the same serovar. They are determined by the presence or absence of enzymes and hence are genetically determined. Biovars may serve as markers and be of interest epidemiologically (for example, the xylose^{+} and xylose^{-} character of *S. typhi*).

Phagovars are determined by the sensitivity of cultures to a series of bacteriophages at appropriate dilutions. Phage typing of *S. typhi* and other salmonellae which possess the Vi antigen ("*S. hirschfeldii*" and rarely "*S. dublin*") is based on a series of adapted phages from phage Vi-II of Craigie and Yen (1938). Phage typing of "*S. schottmuelleri*" (Felix and Callow, 1943) and *S. typhimurium* (Anderson, 1964) uses a different series of phages. Analogous methods have been proposed for other serovars of *Salmonella*, some of them making use of the lysogenicity of the strains.

Other subdivisions of the serovars may be made on the basis of the production of, or the sensitivity to, bacteriocins and on the basis of the resistance to antibiotics.

Genetics. The genetic map of *S. typhimurium* (Sanderson and Hartman, 1978) is not very different from that of *E. coli* K12 (Bachmann and Low, 1980). Hfr strains of *Salmonella* may be selected after F plasmid transfer. Conjugative chromosomal transfer may occur from *Salmonella* to *E. coli*, from *E. coli* to *Salmonella*, and from one serovar of *Salmonella* to another. Chromosomal genes responsible for O, Vi and H antigens can be transferred from one genus to the other (Iino and Lederberg, 1964). Crosses may be used to localize the regions of the bacterial chromosome which specify avirulence for mice (Krishnapillai and Baron, 1964) or to study the role of O antigen factors in the virulence of *Salmonella* (Mäkelä et al., 1973).

As for other *Enterobacteriaceae*, salmonellae may harbor "foreign" replicons—temperate phages or plasmids that may code for antibiotic resistance or for metabolic characters commonly used in diagnostic identification, e.g. lactose or sucrose fermentation (Le Minor et al., 1973, 1974). Thus it is unwise to exclude *Salmonella* solely on the basis of a positive lactose or sucrose reaction. It is also more difficult to identify salmonellae when a pleiotrophic mutation occurs, such as one that simultaneously affects nitrate, tetrathionate, and thiosulfate reductase as well as hydrogenlyase (Le Minor, et al., 1969).

About 5% of *Salmonella* strains produce bacteriocins active against *Escherichia coli*, *Shigella* and/or *Salmonella* (Fredericq, 1948). Most of these bacteriocins adsorb to the same receptor as that for colicins B, E_1, E_2 or I. *Salmonella* bacteriocins differ from colicins *sensu stricto* by their activity spectra on colicin indicator strains. Some of these *Salmonella* bacteriocins are not even active against colicin indicator strains but are active against *Salmonella* strains only (Hamon and Péron, 1966).

Susceptibility to the 01 phage. Most strains of the genus *Salmonella* are susceptible to the 01 phage of Felix and Callow (1943); this phage is highly specific for *Salmonella*, lysing more than 98% of the strains studied in routine *Salmonella* diagnosis (Kallings, 1967). Whereas the majority of strains of *Salmonella* "subgenus" I and II of diphasic "subgenus" III are lysed (some strains, chiefly of the E group, are resistant), monophasic cultures of "subgenus" III and strains of "subgenus" IV are generally resistant (Bockemühl, 1972). Mutations conferring resistance to 01 phage have been studied by Lindberg (1969), McPhee et al. (1975) and Hudson (1978).

A *Salmonella* phage which attacks only flagellated bacteria was isolated by Sertic and Boulgakov (1936). Sensitivity to this phage depends on the H antigen. For example, bacteria with antigens of the "g" complex are resistant (Meynell, 1961).

Pathogenicity. Salmonella serovars may be strictly adapted to one particular host (these serovars are auxotrophic), may be ubiquitous (found in a large number of animal species), or may be of still unknown pathogenicity.

Serovars adapted to man (e.g., *S. typhi*, "*S. paratyphi A*," "*S. sendai*") usually cause grave diseases with septicemia-typhoidic syndrome. They are not pathogenic for other animal species. Salmonellosis is transmitted from man to man, without an intermediate host, through fecal contamination of water and food. The incidence is higher in developing countries with poor hygiene. Other serovars are adapted to one animal species; e.g., "*S. abortusovis*" is adapted to sheep and is a major cause of abortion in ewes, whereas "*S. typhisuis*" and "*S. gallinarum*" ("*S. pullorum*") are adapted to swine and poultry, respectively.

Ubiquitous *Salmonella* serovars (e.g., *S. typhimurium*) are mostly responsible for food-borne infections. It is necessary to ingest a sufficiently high number of bacteria (10^8 to 10^9) to express clinical symptoms. Salmonellosis of newborns and infants (who are more susceptible to infections than adults) presents diverse clinical symptoms, from a grave typhoid-like illness with septicemia to a mild or asymptomatic infection. In pediatric wards the infection is transmitted by the hands of personnel.

The entrance of a serovar into a food chain may be the origin of its implantation in a country. For example, many countries have become infected with "*S. hadar*" introduced by imported turkeys, or by "*S. agona*" introduced by fish meal imported from South America.

After recovery from a clinical case of salmonellosis, some patients—although asymptomatic—remain carriers for weeks, months, or years (i.e. continue to eliminate salmonellae in feces). Carriage contributes to the dissemination of salmonellosis, especially if the diagnosis of the

(Text continues on p. 445)

Table 5.11.
Antigenic formulae of the serovars of the genus **Salmonella**[a]

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
	Group 02 (A)		
S. paratyphi A	1,2,12	a	[1,5]
S. nitra	2,12	g,m	–
S. kiel	1,2,12	g,p	–
	Group 04 (B)		
S. kisangani	1,4,[5],12	a	1,2
S. hessarek	4,12,27	a	1,5
S. fulica	4,[5],12	a	1,5
S. arechavaleta	4,[5],12	a	[1,7]
S. bispebjerg	1,4,[5],12	a	e,n,x
S. tinda	1,4,12,27	a	e,n,z_{15}
S. II *makoma*	4,[5],12	a	–
S. nakura	1,4,12,27	a	z_6
S. paratyphi B[b]	1,4,[5],12	b	1,2
S. limete	1,4,12,27	b	1,5
S. canada	4,12	b	1,6
S. uppsala	4,12,27	b	1,7
S. abony	1,4,[5],12,27	b	e,n,x
S. abortusbovis	1,4,12,27	b	e,n,x
S. II *sofia*	1,4,12,27	b	[e,n,x]
S. wagenia	1,4,12,27	b	e,n,z_{15}
S. wien	1,4,12,27	b	1,w
S. schleissheim	4,12,27	b	–
S. legon	1,4,12,27	c	1,5
S. abortusovis	4,12	c	1,6
S. altendorf	4,12,27	c	1,7
S. jericho	1,4,12,27	c	e,n,z_{15}
S. hallfold	1,4,12,27	c	l,w
S. bury	4,12,27	c	z_6
S. stanley	1,4,[5],12,27	d	1,2
S. eppendorf	1,4,12,27	d	1,5
S. brezany	1,4,12,27	d	1,6
S. schwarzengrund	1,4,12,27	d	1,7
S. II *kluetjenfelde*	4,12	d	e,n,x
S. sarajane	4,[5],12,27	d	e,n,x
S. duisburg	1,4,12,27	d	e,n,z_{15}
S. salinatis	4,12	d,e,h	d,e,n,z_{15}
S. mons	1,4,12,27	d	l,w
S. ayinde	1,4,12,27	d	z_6
S. saintpaul	1,4,[5],12	e,h	1,2
S. reading	1,4,[5],12	e,h	1,5
S. eko	4,12	e,h	1,6
S. kaapstad	4,12	e,h	1,7
S. chester	1,4,[5],12	e,h	e,n,x
S. sandiego	4,[5],12	e,h	e,n,z_{15}
S. II *makumira*	1,4,12,27	e,n,x	1,[5],7
S. derby	1,4,[5],12	f,g	[1,2]
S. agona	1,4,12	f,g,s	–
S. II	1,4,[5],12	f,g,t	z_6:z_{42}
S. essen	4,12	g,m	–
S. hato	4,[5],12	g,m,s	–

[a] Supplemented by the formulae approved up to the end of 1980 and including those for *S. arizonae* ("*Arizona*"). A supplement to the Kauffmann-White scheme, describing the formulae and biochemical characteristics of new *Salmonella* serovars, is published annually in the Annales de Microbiologie (Institut Pasteur), Paris.

Symbols: [ ], may be absent; ( ), not well developed (weakly agglutinable). The symbols for somatic factors whose presence is connected with phage conversion are underlined (e.g. 6,14,18). They are present only if the culture is lysogenized by the corresponding converting phage. These factors are mentioned in the table for serovars in which they were found. It is probable that most, if not all, serovars in a group could be converted by these bacteriophages.

All the "subgenus" I serovars bear a name (e.g. *S. paratyphi A*). "Subgenus" II serovars have the designation "*S.* II," and atypical members of "subgenus" II are designated "S. (II)." The serovars belong to this "subgenus" and those which were described before the Moscow International Congress (1966) bear a name (e.g. S. II *sofia*). Those described subsequently are designated solely by their antigenic formula (e.g. *S.* II 1,4,12,27:z:1,5). Members of "subgenus" III are designated "*S.* III." The serovars of this "subgenus" appear in the table with the name *S. arizonae*, followed by the formula according to the symbols used in the Kauffmann-White scheme and, in parentheses, the formula according to Edwards, Fife and Ewing. The extent to which these two formulae correspond has been established by Dr. R. Rohde. Members of "subgenus" IV are designated "*S.* IV." Members of "subgenus" V are designated "*S.* V" and a name (e.g. *S.* V *bongor*). This is provisional because initially they were considered as atypical strains of "subgenus" I.

Groups C_4, E_2 and E_3 are retained in this table, although it has been shown that the serovars belonging to them are, respectively, those of groups C_1 lysogenized by phage 14 (6, 7), and E_1 lysogenized by ϵ_{15} or $\epsilon_{15} + \epsilon_{34}$. No further serovars have been added to these groups, which are retained provisionally.

[b] Biovar *d*-tartrate positive is often called var. *java*.

[c] May possess an R-phase H antigen: z_{43}.

[d] May possess an R-phase H antigen: z_{40}.

[e] May possess an R-phase H antigen: 1,11; z_{37}, z_{49}.

[f] May possess an R-phase H antigen: z_{50}.

[g] May possess an R-phase H antigen: z_{47}; z_{50}.

[h] May possess an R-phase H antigen: z_{50}.

[i] May possess an R-phase H antigen: z_{45}.

[j] May possess an R-phase H antigen: j; z_{66}.

[k] May possess an R-phase H antigen: z_{40}.

[l] May possess an R-phase H antigen: 1,13.

[m] The serovars of this group also contain the factors 0:3 and (10), the latter not very well developed. They can be lysogenized by phages ϵ_{15} and ϵ_{34} and in the case of double lysogenization become strongly agglutinable, like strains of group E_3, by antisera against 0:34 and 0:12_2.

[n] May possess an R-phase H antigen: z_{45}.

[o] May possess an R-phase H antigen: z_{48}.

[p] May possess an R-phase H antigen: z_{37}.

[q] May possess an R-phase H antigen: z_{45}.

[r] May possess an R-phase H antigen: z_{45}

[s] May possess an R-phase H antigen: z_{49}.

[t] May possess an R-phase H antigen: z_{37}.

[u] May possess an R-phase H antigen: z_{27}; z_{34}; z_{43}; z_{45}; z_{46}.

[v] May possess an R-phase H antigen: z_{59}.

[w] May possess an R-phase H antigen: z_{37}.

[x] May possess an R-phase H antigen: z_{37}; z_{43}.

[y] May possess an R-phase H antigen: z_{45}.

[z] May possess an R-phase H antigen: z_{33}; z_{49}.

[aa] The antigenic factor described for this strain as *Ar.* 32a,32c is very different from other factors H_{32} of *Arizona* 32a,32b. Factor 32b is strongly related to *Salmonella* H factor c.

[bb] May possess an R-phase H antigen: z_{59}.

[cc] May possess an R-phase H antigen: z_{58}.

[dd] May possess an R-phase H antigen: z_{27}.

[ee] May possess an R-phase H antigen: z_{50}.

[ff] May possess an R-phase H antigen: z_{45}.

[gg] May possess an R-phase H antigen: z_{50}.

[hh] May possess an R-phase H antigen: z_{50}.

[ii] May possess an R-phase H antigen: z_{58}.

[jj] May possess an R-phase H antigen: z_{50}.

[kk] May possess an R-phase H antigen: 2_{50}

[ll] May possess an R-phase H antigen: z_{45}.

[mm] May possess an R-phase H antigen: z_{47}; z_{50}.

[nn] May possess an R-phase H antigen: z_{58}.

[oo] May possess an R-phase H antigen: z_{50}.

[pp] This group is not homogeneous and certain serovars possess factors other than 54. Moreover, factor 054 (which has some antigenic resemblance to 042) can be lost by certain serovars: *S. tonev*, which possess factor 21, then becomes similar to *S. minnesota*, *S. uccle* retains factor 3 on this segregation. *S. poeseldorf*, which posessses factors 8,20, becomes similar to *S. kentucky*, and *S. ochsenwerder*, which possesses factors $6_1,6_2,7$, becomes similar to *S. thompson*. *S. steinwerder* can, moreover, be converted by phage ϵ34 and acquire factors 34 and 12_3.

[qq] May possess an R-phase H antigen: z_{47}.

[rr] The group 064 is combined with the group 048 (Winkle, I. 1976, Ann. Microbiol. (Inst. Pasteur) *127B*: 463–472.)

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. II *caledon*	1,4,12,27	g,m,[s],t	e,n,x
S. II *bechuana*	1,4,12,27	g,[m],t	[1,5]
S. II	4,12	g,m,t	z_{39}
S. california	4,12	g,m,t	–
S. kingston[c]	1,4,[5],12,27	g,s,t	[1,2]
S. budapest	1,4,12,27	g,t	–
S. travis	4,[5],12	g,z_{51}	1,7
S. tennyson	4,5,12	g,z_{51}	e,n,z_{15}
S. II	4,12	g,z_{62}	–
S. banana	4,[5],12	m,t	1,5
S. typhimurium	1,4,[5],12	i	1,2
S. lagos	1,4,[5],12	i	1,5
S. agama	4,12	i	1,6
S. tsevie	4,12	i	e,n,z_{15}
S. gloucester	1,4,12,27	i	l,w
S. massenya	1,4,12,27	k	1,5
S. neumuenster	1,4,12,27	k	1,6
S. II	1,4,12,27	k	1,6
S. ljubljana	4,12,27	k	e,n,x
S. texas	4,[5],12	k	e,n,z_{15}
S. fyris	4[5],12	l,v	1,2
S. azteca	4,[5],12,27	l,v	1,5
S. clackamas	4,12	l,v	1,6
S. bredeney[d]	1,4,12,27	l,v	1,7
S. kimuenza	1,4,12,27	l,v	e,n,x
S. II	1,4,12,27	l,v	e,n,x
S. brandenburg	1,4,12	l,v	e,n,z_{15}
S. II	1,4,12,27	l,v	z_{39}
S. mono	4,12	l,w	1,5
S. togo	4,12	l,w	1,6
S. II *kilwa*	4,12	l,w	e,n,x
S. ayton	1,4,12,27	l,w	z_6
S. kunduchi	1,4,[5],12,27	l,[z_{13}], z_{28}	1,2
S. tyresoe	4,12	l,[z_{13}],z_{28}	1,5
S. kubacha	1,4,12,27	l,z_{13},z_{28}	1,7
S. kano	1,4,12,27	l,z_{13},z_{28}	e,n,x
S. vom	1,4,12,27	l,z_{13},z_{28}	e,n,z_{15}
S. reinickendorf	4,12	l,z_{28}	e,n,x
S. II	4,12	l,z_{28}	–
S. heidelberg	1,4,[5],12	r	1,2
S. bradford	4,12,27	r	1,5
S. remo	1,4,12,27	r	1,7
S. bochum	4,[5],12	r	l,w
S. southampton	1,4,12,27	r	z_6
S. drogana	1,4,12,27	r,i	e,n,z_{15}
S. africana	4,12	r,i	l,w
S. coeln	4,[5],12	y	1,2
S. trachau	4,12,27	y	1,5
S. teddington	1,4,12,27	y	1,7
S. ball	1,4,[5],12,27	y	e,n,x
S. jos	1,4,12,27	y	e,n,z_{15}
S. kamoru	4,12,27	y	z_6
S. shubra	4,[5],12	z	1,2
S. kiambu	4,12	z	1,5
S. II	1,4,12,27	z	1,5
S. indiana	1,4,12	z	1,7
S. neftenbach	4,12	z	e,n,x
S. II *nordenham*	1,4,12,27	z	e,n,x
S. koenigstuhl	1,4,12	z	e,n,z_{15}
S. preston	1,4,12	z	l,w
S. entebbe	1,4,12,27	z	z_6
S. stanleyville	1,4,[5],12,27	z_4,z_{23}	[1,2]
S. kalamu	4,[5],12	z_4,z_{24}	[1,5]
S. haifa	1,4,[5],12	z_{10}	1,2
S. ituri	1,4,12	z_{10}	1,5
S. tudu	4,12	z_{10}	1,6
S. albert	4,12	z_{10}	e,n,x
S. tokoin	4,12	z_{10}	e,n,z_{15}
S. mura	1,4,12	z_{10}	l,w
S. fortune	1,4,12,27	z_{10}	z_6
S. vellore	1,4,12,27	z_{10}	z_{35}
S. brancaster	1,4,12,27	z_{29}	–
S. II *helsinki*	1,4,12	z_{29}	[e,n,x]
S. pasing	4,12	z_{35}	1,5
S. tafo	1,4,12,27	z_{35}	1,7
S. sloterdijk	1,4,12,27	z_{35}	z_6
S. yaounde	1,4,12,27	z_{35}	e,n,z_{15}
S. tejas	4,12	z_{36}	–
S. wilhelmsburg	1,4,[5],12,27	z_{38}	–
S. II *durbanville*	1,4,12,27	z_{39}	1,[5],7
S. thayngen	1,4,12,27	z_{41}	1,(2),5
S. abortusequi	4,12	–	e,n,x
	Group 06,7 (C_1)		
	(The strains of this group may be lysogenized by phage 14 → 6,7,14)		
S. sanjuan	6,7	a	1,5
S. umhlali	6,7	a	1,6
S. austin	6,7	a	1,7
S. oslo	6,7	a	e,n,x
S. denver	6,7	a	e,n,z_{15}
S. coleypark	6,7	a	l,w
S. II	6,7	a	z_6
S. II *calvinia*	6,7	a	z_{42}
S. brazzaville	6,7	b	1,2
S. edinburg	6,7	b	1,5
S. adime	6,7	b	1,6
S. koumra	6,7	b	1,7
S. georgia	6,7	b	e,n,z_{15}
S. II *bloemfontein*	6,7	b	[e,n,x]:z_{42}
S. ohio	6,7	b	l,w
S. leopoldville	6,7	b	z_6
S. kotte	6,7	b	z_{35}
S. II	6,7	b	z_{39}
S. paratyphi C	6,7,[Vi]	c	1,5
S. choleraesuis	6,7	[c]	1,5
S. typhisuis	6,7	c	1,5
S. birkenhead	6,7	c	1,6
S. kisii	6,7	d	1,2
S. isangi	6,7	d	1,5
S. kivu	6,7	d	1,6
S. kambole	6,7	d	1,7
S. II	6,7	d	1,7
S. amersfoort	6,7	d	e,n,x
S. gombe	6,7	d	e,n,z_{15}
S. livingstone	6,7	d	l,w
S. wil	6,7	d	l,z_{13},z_{28}
S. larochelle	6,7	e,h	1,2
S. lomita	6,7	e,h	1,5
S. norwich	6,7	e,h	1,6
S. braenderup	6,7	e,h	e,n,z_{15}
S. rissen	6,7	f,g	–
S. eingedi	6,7	f,g,t	1,2,7
S. afula	6,7	f,g,t	e,n,x
S. montevideo	6,7	g,m,[p],s	[1,2,7]
S. II	6,7	g,m,[s],t	e,n,x
S. II	6,7	(g),m,[s],t	1,5
S. II	6,7	g,m,s,t	z_{42}
S. othmarschen	6,7	g,m,[t]	–
S. menston	6,7	g,s,t	[1,6]
S. II	6,7	g,t	e,n,x:z_{42}
S. riggil	6,7	g,t	–
S. alamo	6,7	g,z_{51}	1,5
S. haelsingborg	6,7	m,p,t,[u]	–
S. oranienburg	6,7	m,t	–
S. augustenborg	6,7	i	1,2
S. oritamerin	6,7	i	1,5
S. garoli	6,7	i	1,6
S. lika	6,7	i	1,7
S. athinai	6,7	i	e,n,z_{15}

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. norton	6,7	i	l,w
S. galiema	6,7	k	1,2
S. thompson	6,7	k	1,5
S. daytona	6,7	k	1,6
S. baiboukoum	6,7	k	1,7
S. singapore	6,7	k	e,n,x
S. escanaba	6,7	k	e,n,z_{15}
S. III *arizonae* (Ar. 27:22:31:37)	6,7	(k)	z:[z_{55}]
S. II	6,7	k	[z_6]
S. concord	6,7	l,v	1,2
S. irumu	6,7	l,v	1,5
S. mkamba	6,7	l,v	1,6
S. kortrijk	6,7	l,v	1,7
S. bonn	6,7	l,v	e,n,x
S. potsdam	6,7	l,v	e,n,z_{15}
S. gdansk	6,7	l,v	z_6
S. III *arizonae* (Ar. 27:23:25)	6,7	l,v	z_{53}
S. gabon	6,7	l,w	1,2
S. colorado	6,7	l,w	1,5
S. II	6,7	l,w	1,5,7
S. nessziona	6,7	l,z_{13}	1,5
S. kenya	6,7	l,z_{13}	e,n,x
S. neukoelln	6,7	l,z_{13},[z_{28}]	e,n,z_{15}
S. makiso	6,7	l,z_{13},z_{28}	z_6
S. II *heilbron*	6,7	l,z_{28}	1,5:[z_{42}]
S. virchow	6,7	r	1,2
S. infantis[e]	6,7	r	1,5
S. nigeria	6,7	r	1,6
S. colindale	6,7	r	1,7
S. papuana	6,7	r	e,n,z_{15}
S. grampian	6,7	r	l,w
S. richmond	6,7	y	1,2
S. bareilly	6,7	y	1,5
S. oyonnax	6,7	y	1,6
S. gatow	6,7	y	1,7
S. hartford[f]	6,7	y	e,n,x
S. mikawasima[g]	6,7	y	e,n,z_{15}
S. II *tosamanga*	6,7	z	1,5
S. oakland	6,7	z	1,6[7]
S. cayar	6,7	z	e,n,x
S. businga	6,7	z	e,n,z_{15}
S. bruck	6,7	z	l,w
S. II	6,7	z	z_6
S. II	6,7	z	z_{39}
S. II *oysterbeds*	6,7	z	z_{42}
S. obogu	6,7	z_4,z_{23}	1,5
S. aequatoria	6,7	z_4,z_{23}	e,n,z_{15}
S. goma	6,7	z_4,z_{23}	z_6
S. IV *roterberg*	6,7	z_4,z_{23}	–
S. somone	6,7	z_4,z_{24}	–
S. IV *kralendyk*	6,7	z_4,z_{24}	–
S. II *cape*	6,7	z_6	1,7
S. menden	6,7	z_{10}	1,2
S. inganda	6,7	z_{10}	1,5
S. eschweiler	6,7	z_{10}	1,6
S. ngili	6,7	z_{10}	1,7
S. djugu	6,7	z_{10}	e,n,x
S. mbandaka	6,7	z_{10}	e,n,z_{15}
S. redba	6,7	z_{10}	z_6
S. II	6,7	z_{10}	z_{35}
S. tennessee	6,7	z_{29}	[1,2,7]
S. II	6,7	z_{29}	–
S. palime	6,7	z_{35}	e,n,z_{15}
S. II *bacongo*	6,7	z_{36}	z_{42}
S. IV *argentina*	6,7	z_{36}	–
S. rumford	6,7	z_{38}	1,2
S. lille	6,7	z_{38}	–
S. II *gilbert*	6,7	z_{39}	1,5,7
S. II	6,7	z_{41}	1,7
S. hillsborough	6,7	z_{41}	l,w
S. tamilnadu	6,7	z_{41}	z_{35}
S. II *sullivan*	6,7	z_{42}	1,7
S. II	6,7	z_{42}	e,n,x:1,6
S. III *arizonae* (Ar.27:–:30)	6,7	–	1,6
	Group 06,8 (C_2)		
S. doncaster	6,8	a	1,5
S. curacao	6,8	a	1,6
S. nordufer	6,8	a	1,7
S. narashino	6,8	a	e,n,x
S. II	6,8	a	e,n,x
S. leith	6,8	a	e,n,z_{15}
S. II *tulear*	6,8	a	z_{62}
S. skansen	6,8	b	1,2
S. nagoya	6,8	b	1,5
S. stourbridge	6,8	b	1,6
S. eboko	6,8	b	1,7
S. gatuni	6,8	b	e,n,x
S. presov	6,8	b	e,n,z_{15}
S. bukuru	6,8	b	l,w
S. banalia	6,8	b	z_6
S. wingrove	6,8	c	1,2
S. utah	6,8	c	1,5
S. bronx	6,8	c	1,6
S. belfast	6,8	c	1,7
S. belem	6,8	c	e,n,x
S. quiniela	6,8	c	e,n,z_{15}
S. muenchen	6,8	d	1,2
S. manhattan	6,8	d	1,5
S. sterrenbos	6,8	d	e,n,x
S. herston	6,8	d	e,n,z_{15}
S. II	6,8	d	z_6:z_{42}
S. newport[h]	6,8	e,h	1,2
S. kottbus	6,8	e,h	1,5
S. cremieu	6,8	e,h	1,6
S. tshiongwe	6,8	e,h	e,n,z_{15}
S. sandow	6,8	f,g	e,n,z_{15}
S. chincol	6,8	g,m,[s]	[e,n,x]
S. II	6,8	g,m,t	[e,n,x]
S. nanergou	6,8	g,s,t	–
S. II *baragwanath*	6,8	m,t	1,5
S. II *germiston*	6,8	m,t	e,n,x
S. bassa	6,8	m,t	–
S. lindenburg	6,8	i	1,2
S. takoradi	6,8	i	1,5
S. warnow	6,8	i	1,6
S. malmoe	6,8	i	1,7
S. bonariensis	6,8	i	e,n,x
S. aba	6,8	i	e,n,z_{15}
S. cyprus	6,8	i	l,w
S. blockley	6,8	k	1,5
S. schwerin	6,8	k	e,n,x
S. charlottenburg	6,8	k	e,n,z_{15}
S. litchfield	6,8	l,v	1,2
S. loanda	6,8	l,v	1,5
S. manchester	6,8	l,v	1,7
S. holcomb	6,8	l,v	e,n,x
S. II	6,8	l,v	e,n,x
S. edmonton	6,8	l,v	e,n,z_{15}
S. fayed	6,8	l,w	1,2
S. hiduddify	6,8	l,z_{13},z_{28}	1,5
S. breukelen	6,8	l,z_{13},[z_{28}]	e,n,z_{15}
S. bovismorbificans	6,8	r	1,5
S. akanji	6,8	r	1,7
S. hidalgo	6,8	r	e,n,z_{15}
S. goldcoast	6,8	r	l,w
S. tananarive	6,8	y	1,5

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. bulgaria	6,8	y	1,6
S. II	6,8	y	1,6:z_{42}
S. inchpark	6,8	y	1,7
S. praha	6,8	y	e,n,z_{15}
S. mowanjum	6,8	z	1,5
S. II	6,8	z	1,5
S. kalumburu	6,8	z	e,n,z_{15}
S. kuru	6,8	z	l,w
S. lezennes	6,8	z_4,z_{23}	1,7
S. chailey	6,8	z_4,z_{23}	e,n,z_{15}
S. duesseldorf	6,8	z_4,z_{24}	–
S. tallahassee	6,8	z_4,z_{32}	–
S. zerifin	6,8	z_{10}	1,2
S. mapo	6,8	z_{10}	1,5
S. cleveland	6,8	z_{10}	1,7
S. hadar	6,8	z_{10}	e,n,x
S. glostrup	6,8	z_{10}	e,n,z_{15}
S. wippra	6,8	z_{10}	z_6
S. II	6,8	z_{29}	1,5
S. uno	6,8	z_{29}	[e,n,z_{15}]
S. yarm	6,8	z_{35}	1,2
S. aesch	6,8	z_{60}	1,2
	Group 08 (C_3)		
S. be	8,20	a	–
S. djelfa	8	b	1,2
S. korbol	8,20	b	1,5
S. sanga	8	b	1,7
S. konstanz	8	b	e,n,x
S. shipley	8,20	b	e,n,z_{15}
S. tounouma	8,20	b	z_6
S. alexanderpolder	8	c	l,w
S. santiago	8,20	c	e,n,x
S. tado	8,20	c	z_6
S. virginia	8	d	1,2
S. yovokome	8	d	1,5
S. labadi	8,20	d	z_6
S. bardo	8	e,h	1,2
S. ferruch	8	e,h	1,5
S. atakpame	8,20	e,h	1,7
S. rechovot	8,20	e,h	z_6
S. emek	8,20	g,m,s	–
S. reubeuss	8,20	g,m,t	–
S. alminko	8,20	g,s,t	–
S. yokoe	8	m,t	–
S. bargny	8,20	i	1,5
S. kentucky	8,20	i	z_6
S. haardt	8	k	1,5
S. pakistan	8	l,v	1,2
S. amherstiana	8	l,v	1,6
S. hindmarsh	8,20	r	1,5
S. cocody	8,20	r,i	e,n,z_{15}
S. brikama	8,20	r,i	l,w
S. altona	8,20	r,[i]	z_6
S. giza	8,20	y	1,2
S. brunei	8,20	y	1,5
S. alagbon	8	y	1,7
S. sunnycove	8	y	e,n,x
S. kralingen	8,20	y	z_6
S. bellevue	8	z_4,z_{23}	1,7
S. dabou	8,20	z_4,z_{23}	l,w
S. corvallis	8,20	z_4,z_{23}	[z_6]
S. albany[i]	8,20	z_4,z_{24}	–
S. bazenheid	8,20	z_{10}	1,2
S. paris	8,20	z_{10}	1,5
S. istanbul	8	z_{10}	e,n,x
S. chomedey	8	z_{10}	e,n,z_{15}
S. molade	8,20	z_{10}	z_6
S. II	8	z_{29}	e,n,x:z_{42}
S. tamale	8,20	z_{29}	[e,n,z_{15}]
S. angers	8,20	z_{35}	z_6
S. apeyeme	8,20	z_{35}	–
S. diogoye	8,20	z_{41}	z_6
	Group 06,7,14 (C_4)		
	(*Salmonella* serovars of group C_1 lysogenized by "phage 14")		
S. lockleaze	6,7,14	b	e,n,x
S. nienstedten	6,7,14	b	[l,w]
S. hissar	6,7,14	c	1,2
S. kaduna	6,7,14	c	e,n,z_{15}
S. omderman	6,7,14	d	e,n,x
S. eimsbuettel	6,7,14	d	l,w
S. nieukerk	6,7,14	d	z_6
S. ardwick	6,7,14	f,g	–
S. thielallee	6,7,14	m,t	–
S. gelsenkirchen	6,7,14	l,v	z_6
S. jerusalem	6,7,14	z_{10}	l,w
S. bornum	6,7,14	z_{38}	–
S. III *arizonae* (Ar. 27:45:30)	6,7,14	z_{39}	1,2
	Group 09, 12 (D_1)		
S. sendai	1,9,12	a	1,5
S. miami	1,9,12	a	1,5
S. II	9,12	a	1,5
S. os	9,12	a	1,6
S. saarbruecken	1,9,12	a	1,7
S. lomalinda	1,9,12	a	e,n,x
S. II	1,9,12	a	e,n,x
S. durban	9,12	a	e,n,z_{15}
S. II	9,12	a	z_{39}
S. onarimon	1,9,12	b	1,2
S. frintrop	1,9,12	b	1,5
S. II *mjimwema*	1,9,12	b	e,n,x
S. II *blankenese*	1,9,12	b	z_6
S: II *suederelbe*	1,9,12	b	z_{39}
S. goeteborg	9,12	c	1,5
S. ipeko	9,12	c	1,6
S. elokate	9,12	c	1,7
S. alabama	9,12	c	e,n,z_{15}
S. ridge	9,12	c	z_6
S. ndolo	1,9,12	d	1,5
S. tarshyne	9,12	d	1,6
S. II *rhodesiense*	9,12	d	e,n,x
S. zega	9,12	d	z_6
S. jaffna	1,9,12	d	z_{35}
S. typhi	9;12,[Vi]	d	–
S. bournemouth	9,12	e,h	1,2
S. eastbourne	1,9,12	e,h	1,5
S. israel	9,12	e,h	e,n,z_{15}
S. II *lindrick*	9,12	e,n,x	1,[5],7
S. II	9,12	e,n,x	1,6
S. berta	1,9,12	f,g,t	–
S. enteritidis	1,9,12	g,m	[1,7]
S. blegdam	9,12	g,m,q	–
S. II	1,9,12	g,m,[s],t	[1,5]:[z_{42}]
S. II *kuilsrivier*	1,9,12	g,m,s,t	e,n,x
S. dublin	1,9,12[Vi]	g,p	–
S. naestved	1,9,12	g,p,s	–
S. rostock	1,9,12	g,p,u	–
S. moscow	9,12	g,q	–
S. II *neasden*	9,12	g,s,t	e,n,x
S. newmexico	9,12	g,z_{51}	1,5
S. II	1,9,12	g,z_{62}	–
S. antarctica	9,12	g,z_{63}	–
S. II	9,12	m,t	e,n,x
S. pensacola	1,9,12	m,t	–
S. seremban	9,12	i	1,5
S. claibornei	1,9,12	k	1,5
S. goverdhan	9,12	k	1,6
S. mendoza	9,12	l,v	1,2
S. panama	1,9,12	l,v	1,5
S. kapemba[k]	9,12	l,v	1,7

Table 5.11 (*Continued*)

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. II	9,12	l,v	e,n,x
S. goettingen	9,12	l,v	e,n,z_{15}
S. II	9,12	l,v	z_{39}
S. victoria	1,9,12	l,w	1,5
S. II *daressalaam*	1,9,12	l,w	e,n,x
S. itami	9,12	l,z_{13}	1,5
S. miyazaki	9,12	l,z_{13}	1,7
S. napoli	1,9,12	l,z_{13}	e,n,x
S. javiana[l]	1,9,12	l,z_{28}	1,5
S. II	9,12	l,z_{28}	e,n,x
S. jamaica	9,12	r	1,5
S. camberwell	9,12	r	1,7
S. campinense	9,12	r	e,n,z_{15}
S. lome	9,12	r	z_6
S. lawndale	1,9,12	z	1,5
S. kimpese	9,12	z	1,6
S. II *stellenbosch*	1,9,12	z	1,7
S. II *angola*	1,9,12	z	z_6
S. II *hueningen*	9,12	z	z_{39}
S. wangata	1,9,12	z_4,z_{23}	[1,7]
S. portland	9,12	z_{10}	1,5
S. II *canastel*	9,12	z_{29}	1,5
S. II	1,9,12	z_{29}	e,n,x
S. penarth	9,12	z_{35}	z_6
S. elomrane	1,9,12	z_{38}	–
S. II *wynberg*	1,9,12	z_{39}	1,7
S. ottawa	1,9,12	z_{41}	1,5
S. gallinarum-pullorum	1,9,12	–	–
	Group 09,46 (D_2)[m]		
S. baildon	9,46	a	e,n,x
S. doba	9,46	a	e,n,z_{15}
S. zadar	9,46	b	1,6
S. worb	9,46	b	e,n,x
S. II *lundby*	9,46	b	e,n,x
S. bamboye	9,46	b	l,w
S. linguere	9,46	b	z_6
S. itutaba	9,46	c	z_6
S. ontario	9,46	d	1,5
S. quentin	9,46	d	1,6
S. strasbourg	9,46	d	1,7
S. olten	9,46	d	e,n,z_{15}
S. plymouth	9,46	d	z_6
S. bergedorf	9,46	e,h	1,2
S. guerin	9,46	e,h	z_6
S. II	9,46	e,n,x	1,5,7
S. wernigerode	9,46	f,g	–
S. hillingdon	9,46	g,m	–
S. II *duivenhoks*	9,46	g,m,s,t	e,n,x
S. gatesnead	9,46	g,s,t	–
S. II	9,46	m,t	e,n,x
S. sangalkam	9,46	m,t	–
S. mathura	9,46	i	e,n,z_{15}
S. potto	9,46	i	z_6
S. marylebone	9,46	k	1,2
S. cochin	9,46	k	1,5
S. ceyco	9,46	k	z_{35}
S. india	9,46	l,v	1,5
S. geraldton	9,46	l,v	1,6
S. toronto	9,46	l,v	e,n,x
S. shoreditch	9,46	r	e,n,z_{15}
S. sokode	9,46	r	z_6
S. benin	9,46	y	1,7
S. mayday	9,46	y	z_6
S. II *haarlem*	9,46	z	e,n,x
S. bambylor	9,46	z	e,n,z_{15}
S. ekotedo	9,46	z_4,z_{23}	–
S. II *maarssen*	9,46	z_4,z_{24}	z_{39},z_{42}
S. lishabi	9,46	z_{10}	1,7
S. inglis	9,46	z_{10}	e,n,x
S. louisiana	9,46	z_{10}	z_6
S. II	9,46	z_{10}	z_6
S. II	9,46	z_{10}	z_{39}
S. ouakam[n]	9,46	z_{29}	–
S. hillegersberg	9,46	z_{35}	1,5
S. basingstoke	9,46	z_{35}	e,n,z_{15}
S. trimdon	9,46	z_{35}	z_6
S. fresno	9,46	z_{38}	–
S. II	9,46	z_{39}	1,7
S. wuppertal	9,46	z_{41}	–
	Group 01,9,12,(46),27 (D_3)		
S. II *zuerich*	1,9,12,(46),27	c	z_{39}
S. II	9,12,(46),27	g,t	e,n,x
S. II	1,9,12,(46),27	l,z_{13},z_{28}	z_{39}
S. II	1,9,12,(46),27	y	z_{39}
S. II	1,9,12,(46),27	z_4,z_{24}	1,5
S. II	1,9,12,(46),27	z_{10}	e,n,x
S. II	1,9,12,(46),27	z_{10}	z_{39}
	Group 03,10 (E_1)		
S. aminatu	3,10	a	1,2
S. goelzau	3,10	a	1,5
S. oxford	3,10	a	1,7
S. masembe	3,10	a	e,n,x
S. II *matroosfontein*	3,10	a	e,n,x
S. galil	3,10	a	e,n,z_{15}
S. II	3,10	a	z_{39}
S. kalina	3,10	b	1,2
S. butantan	3,10	b	1,5
S. allerton	3,10	b	1,6
S. huvudsta	3,10	b	1,7
S. benfica	3,10	b	e,n,x
S. II	3,10	b	e,n,x
S. yaba	3,10	b	e,n,z_{15}
S. epicrates	3,10	b	l,w
S. II	3,10	b	z_{39}
S. gbadago	3,10	c	1,5
S. ikayi	3,10	c	1,6
S. pramiso	3,10	c	1,7
S. agege	3,10	c	e,n,z_{15}
S. anderlecht	3,10	c	l,w
S. okefoko	3,10	c	z_6
S. stormont	3,10	d	1,2
S. shangani	3,10	d	1,5
S. lekke	3,10	d	1,6
S. onireke	3,10	d	1,7
S. souza	3,10	d	e,n,x
S. II	3,10	d	e,n,x
S. madjorio	3,10	d	e,n,z_{15}
S. birmingham	3,10	d	l,w
S. weybridge	3,10	d	z_6
S. maron	3,10	d	z_{35}
S. vejle	3,10	e,h	1,2
S. muenster[o]	3,10	e,h	1,5
S. anatum	3,10	e,h	1,6
S. nyborg	3,10	e,h	1,7
S. newlands	3,10	e,h	e,n,x
S. meleagridis	3,10	e,h	l,w
S. sekondi	3,10	e,h	z_6
S. II *chudleigh*	3,10	e,n,x	1,7
S. regent	3,10	f,g,[s]	[1,6]
S. alfort	3,10	f,g	e,n,x
S. suberu	3,10	g,m	–
S. amsterdam	3,10	g,m,s	–
S. II	3,10	g,m,s,t	–
S. westhampton[p]	3,10	g,s,t	–
S. II *islington*	3,10	g,t	–
S. southbank	3,10	m,t	[1,6]
S. II *stikland*	3,10	m,t	e,n,x
S. cukmere	3,10	i	1,2
S. amounderness	3,10	i	1,5
S. truro	3,10	i	1,7

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. bessi	3,10	i	e,n,x
S. falkensee	3,10	i	e,n,z_{15}
S. yeerongpilly	3,10	i	z_6
S. wimborne	3,10	k	1,2
S. zanzibar	3,10	k	1,5
S. yundum	3,10	k	e,n,x
S. marienthal	3,10	k	e,n,z_{15}
S. newrochelle	3,10	k	l,w
S. nchanga	3,10	l,v	1,2
S. sinstorf	3,10	l,v	1,5
S. london	3,10	l,v	1,6
S. give	3,10	[d]:l,v	1,7
S. II	3,10	l,v	e,n,x
S. ruzizi	3,10	l,v	e,n,z_{15}
S. II *fuhlsbuettel*	3,10	l,v	z_6
S. sinchew	3,10	l,v	z_{35}
S. assinie[q]	3,10	l,w	z_6
S. freiburg	3,10	l,z_{13}	1,2
S. uganda	3,10	l,z_{13}	1,5
S. fallowfield	3,10	l,z_{13},z_{28}	e,n,z_{15}
S. hoghton	3,10	l,z_{13},z_{28}	z_6
S. II	3,10	l,z_{28}	1,5
S. joal	3,10	l,z_{28}	1,7
S. lamin	3,10	l,z_{28}	e,n,x
S. II *westpark*	3,10	l,z_{28}	e,n,x
S. II	3,10	l,z_{28}	z_{39}
S. ughelli	3,10	r	1,5
S. elisabethville	3,10	r	1,7
S. simi	3,10	r	e,n,z_{15}
S. weltevreden	3,10	r	z_6
S. seegefeld	3,10	r,i	1,2
S. dumfries	3,10	r,i	1,6
S. amager[r]	3,10	y	1,2
S. orion	3,10	y	1,5
S. mokola	3,10	y	1,7
S. ohlstedt	3,10	y	e,n,x
S. bolton	3,10	y	e,n,z_{15}
S. langensalza	3,10	y	l,w
S. stockholm	3,10	y	z_6
S. fufu	3,10	z	1,5
S. II *alexander*	3,10	z	1,5
S. huddinge	3,10	z	1,7
S. II *finchley*	3,10	z	e,n,x
S. clerkenwell	3,10	z	l,w
S. landwasser	3,10	z	z_6
S. II *tafelbaai*	3,10	z	z_{39}
S. adabraka	3,10	z_4,z_{23}	[1,7]
S. florian	3,10	z_4,z_{24}	–
S. II	3,10	z_4,z_{24}	–
S. okerara	3,10	z_{10}	1,2
S. lexington[s]	3,10	z_{10}	1,5
S. coquilhatville	3,10	z_{10}	1,7
S. kristianstad	3,10	z_{10}	e,n,z_{15}
S. biafra	3,10	z_{10}	z_6
S. II	3,10	z_{29}	e,n,x
S. jedburgh	3,10	z_{29}	–
S. zongo	3,10	z_{35}	1,7
S. shannon	3,10	z_{35}	l,w
S. cairina	3,10	z_{35}	z_6
S. macallen	3,10	z_{36}	–
S. bolombo	3,10	z_{38}	[z_6]
S. II *mpila*	3,10	z_{38}	z_{42}
S. II *winchester*	3,10	z_{39}	1,7
Group 03,15 (E_2)			
(*Salmonella* serovars of group E_1 lysogenized by phage ϵ_{15})			
S. clichy	3,15	a	1,5
S. rosenthal	3,15	b	1,5
S. westminster	3,15	b	z_{35}
S. pankow	3,15	d	1,5
S. eschersheim	3,15	d	e,n,x
S. goerlitz	3,15	e,h	1,2
S. newhaw	3,15	e,h	1,5
S. newington	3,15	e,h	1,6
S. selandia	3,15	e,h	1,7
S. cambridge	3,15	e,h	l,w
S. drypool	3,15	g,m,s	–
S. II *parow*	3,15	g,m,s,t	–
S. halmstad	3,15	g,s,t	–
S. nancy	3,15	l,v	1,2
S. portsmouth	3,15	l,v	1,6
S. newbrunswick	3,15	l,v	1,7
S. kinshasa	3,15	l,z_{13}	1,5
S. lanka	3,15	r	z_6
S. tuebingen	3,15	y	1,2
S. binza	3,15	y	1,5
S. tournai	3,15	y	z_6
S. manila	3,15	z_{10}	1,5
Group 03,15,34 (E_3)			
(*Salmonella* serovars of group E_1 lysogenized by phages ϵ_{15} and ϵ_{34})			
S. khartoum	3,15,34	a	1,7
S. arkansas	3,15,34	e,h	1,5
S. minneapolis	3,15,34	e,h	1,6
S. wildwood	3,15,34	e,h	l,w
S. canoga	3,15,34	g,s,t	–
S. menhaden	3,15,34	l,v	1,7
S. thomasville	3,15,34	y	1,5
S. illinois	3,15,34	z_{10}	1,5
S. harrisonburg	3,15,34	z_{10}	1,6
Group 01,3,19 (E_4)			
S. juba	1,3,19	a	1,7
S. gwoza	1,3,19	a	e,n,z_{15}
S. gnesta[t]	1,3,19	b	1,5
S. visby	1,3,19	b	1,6
S. tambacounda	1,3,19	b	e,n,x
S. kande	1,3,19	b	e,n,z_{15}
S. broughton	1,3,19	b	l,w
S. accra	1,3,19	b	z_6
S. madiago	1,3,19	c	1,7
S. ahmadi	1,3,19	d	1,5
S. liverpool	1,3,19	d	e,n,z_{15}
S. tilburg	1,3,19	d	l,w
S. niloese	1,3,19	d	z_6
S. vilvoorde	1,3,19	e,h	1,5
S. sanktmarx	1,3,19	e,h	1,7
S. sao	1,3,19	e,h	e,n,z_{15}
S. calabar	1,3,19	e,h	l,w
S. rideau	1,3,19	f,g	–
S. maiduguri	1,3,19	f,g,t	e,n,z_{15}
S. kouka	1,3,19	g,m,[t]	–
S. senftenberg[u]	1,3,19	g,[s],t	–
S. cannstatt	1,3,19	m,t	–
S. stratford	1,3,19	i	1,2
S. machaga	1,3,19	i	e,n,x
S. avonmouth	1,3,19	i	e,n,z_{15}
S. zuilen	1,3,19	i	l,w
S. taksony	1,3,19	i	z_6
S. ngor	1,3,19	l,v	1,5
S. parkroyal	1,3,19	l,v	1,7
S. westerstede	1,3,19	l,z_{13}	[1,2]
S. winterthur	1,3,19	l,z_{13}	1,6
S. lokstedt	1,3,19	l,z_{13},z_{28}	1,2
S. stuivenberg	1,3,19	l,z_{13},z_{28}	1,5
S. bedford	1,3,19	l,z_{13},z_{28}	e,n,z_{15}
S. tomelilla	1,3,19	l,z_{28}	1,7
S. yalding	1,3,19	r	e,n,z_{15}
S. fareham	1,3,19	r,i	l,w
S. gatineau	1,3,19	y	1,5
S. krefeld	1,3,19	y	l,w
S. korlebu	1,3,19	z	1,5
S. lerum	1,3,19	z	1,7

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. schoeneberg	1,3,19	z	e,n,z_{15}
S. carno	1,3,19	z	l,w
S. sambre	1,3,19	z_4,z_{24}	–
S. dallgow	1,3,19	z_{10}	e,n,z_{15}
S. llandoff	1,3,19	z_{29}	[z_6]
S. chittagong	1,3,10,19	b	z_{35}
S. bilu	1,3,10,19	f,g,t	1,(2),7
S. ilugun	1,3,10,19	z_4,z_{23}	z_6
S. dessau	1,3,15,19	g,s,t	–
S. cannonhill	1,3,15,19	y	e,n,x
	Group 011 (F)		
S. gallen	11	a	1,2
S. marseille	11	a	1,5
S. toowong	11	a	1,7
S. luciana	11	a	e,n,z_{15}
S. epinay	11	a	l,z_{13},z_{28}
S. II *glencairn*	11	a	z_6:z_{42}
S. atento	11	b	1,2
S. leeuwarden	11	b	1,5
S. wohlen	11	b	1,6
S. II	11	b	1,7
S. II *srinagar*	11	b	e,n,x
S. pharr	11	b	e,n,z_{15}
S. chiredzi	11	c	1,5
S. gustavia	11	d	1,5
S. chandans	11	d	e,n,x
S. II *montgomery*	11	d,(a)	d,e,n,z_{15}
S. findorff	11	d	z_6
S. chingola	11	e,h	1,2
S. adamstua	11	e,h	1,6
S. redhill	11	e,h	l,z_{13},z_{28}
S. II *grabouw*	11	g,m,s,t	z_{39}
S. IV *mundsburg*	11	g,z_{51}	–
S. II *lincoln*	11	m,t	e,n,x
S. aberdeen	11	i	1,2
S. brijbhumi	11	i	1,5
S. heerlen	11	i	1,6
S. veneziana	11	i	e,n,x
S. pretoria	11	k	1,2
S. abaetetuba	11	k	1,5
S. sharon	11	k	1,6
S. colobane	11	k	1,7
S. kisarawe	11	k	e,n,x[z_{15}]
S. amba	11	k	l,z_{13},z_{28}
S. III *arizonae* (Ar. 17:29:25)	11	k	z_{53}
S. stendal	11	l,v	1,2
S. maracaibo	11	l,v	1,5
S. fann	11	l,v	e,n,x
S. bullbay	11	l,v	e,n,z_{15}
S. III *arizonae* (Ar. 17:23:31)	11	l,v	z
S. III *arizonae* (Ar. 17:23:25)	11	l,v	z_{53}
S. glidji	11	l,w	1,5
S. osnabrueck	11	l,z_{13},z_{28}	e,n,x
S. II *huila*	11	l,z_{28}	e,n,x
S. senegal	11	r	1,5
S. rubislaw	11	r	e,n,x
S. volta	11	r	l,z_{13},z_{28}
S. solt	11	y	1,5
S. jalisco	11	y	1,7
S. herzliya	11	y	e,n,x
S. nyanza	11	z	z_6
S. II *soutpan*	11	z	z_{39}
S. remete	11	z_4,z_{23}	1,6
S. etterbeek	11	z_4,z_{23}	e,n,z_{15}
S. III *arizonae* (Ar. 17:1,2,5:–)	11	z_4,z_{23}	–
S. IV *parera*	11	z_4,z_{23}	–
S. yehuda	11	z_4,z_{24}	–
S. IV	11	z_4,z_{32}	–
S. wentworth	11	z_{10}	1,2
S. straengnaes	11	z_{10}	1,5
S. telhashomer	11	z_{10}	e,n,x
S. lene	11	z_{38}	–
S. maastricht	11	z_{41}	1,2
S. II	11	–	1,5
	Group 013,22 (G_1)		
S. mim	13,22	a	1,6
S. marshall	13,22	a	l,z_{13},z_{28}
S. ibadan	13,22	b	1,5
S. oudwijk	13,22	b	1,6
S. rottnest	1,13,22	b	1,7
S. vaertan	13,22	b	e,n,x
S. bahati	13,22	b	e,n,z_{15}
S. II	1,13,22	b	z_{42}
S. haouaria	13,22	c	e,n,x,z_{15}
S. friedenau	13,22	d	1,6
S. diguel	1,13,22	d	e,n,z_{15}
S. willemstad	1,13,22	e,h	1,6
S. raus	13,22	f,g	e,n,x
S. II	13,22	(f),g,t	–
S. bron	13,22	g,m	[e,n,z_{15}]
S. II *limbe*	1,13,22	g,m,t	[1,5]
S. newyork	13,22	g,s,t	–
S. II *rotterdam*	1,13,22	g,t	1,5
S. washington	13,22	m,t	–
S. II	13,22	k	1,5:z_{42}
S. lovelace	13,22	l,v	1,5
S. borbeck	13,22	l,v	1,6
S. II	13,22	l,z_{28}	1,5
S. tanger	1,13,22	y	1,6
S. poona[o]	1,13,22	z	1,6
S. bristol	13,22	z	1,7
S. tanzania	1,13,22	z	e,n,z_{15}
S. ried	1,13,22	z_4,z_{23}	[e,n,z_{15}]
S. III *arizonae* (Ar. 18:1,2,5)	13,22	z_4,z_3	–
S. roodepoort	1,13,22	z_{10}	1,5
S. II *clifton*	13,22	z_{29}	1,5
S. II *goodwood*	13,22	z_{29}	e,n,x
S. agoueve	13,22	z_{29}	–
S. mampong	13,22	z_{35}	1,6
S. nimes	13,22	z_{35}	e,n,z_{15}
S. leiden	13,22	z_{38}	–
S. II	13,22	z_{39}	1,5,(7)
S. III *arizonae* (Ar. 18:–:–)	13,22	–	–
	Group 013,23 (G_2)		
S. chagoua	1,13,23	a	1,5
S. wyldegreen	13,23	a	l,w
S. II *tygerberg*	1,13,23	a	z_{42}
S. mississippi	1,13,23	b	1,5
S. II *acres*	1,13,23	b	[1,5]:z_4
S. bracknell	13,23	b	1,6
S. ullevi	1,13,23	b	e,n,x
S. durham	13,23	b	e,n,z_{15}
S. handen	1,13,23	d	1,2
S. mishmarhaemek	1,13,23	d	1,5
S. wichita[w]	1,13,23	d	[1,6]
S. grumpensis	13,23	d	1,7
S. II	13,23	d	e,n,x
S. telelkebir	13,23	d	e,n,z_{15}
S. putten	13,23	d	l,w
S. isuge	13,23	d	z_6
S. tschangu	1,13,23	e,h	1,5
S. II *epping*	1,13,23	e,n,x	1,7
S. havana	1,13,23	f,g,[s]	–
S. agbeni	13,23	g,m	–
S. II	13,23	g,m,s,t	1,5
S. II *luanshya*	1,13,23	g,m,[s],t	[e,n,x]
S. congo	13,23	g,m,s,t	–
S. okatie	13,23	g,s,t	–
S. II *gojenberg*	1,13,23	g,t	1,5
S. II	1,13,23	g,t	z_{42}

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. III *arizonae* (Ar. 18:13,14:–)	1,13,23	g,z_{51}	–
S. II *katesgrove*	1,13,23	m,t	1,5
S. II *worcester*	1,13,23	m,t	e,n,x
S. II *boulders*	1,13,23	m,t	z_{42}
S. kintambo	13,23	m,t	–
S. idikan	1,13,23	i	1,5
S. jukestown	13,23	i	e,n,z_{15}
S. kedougou	1,13,23	i	l,w
S. II	13,23	k	z_{41}
S. nanga	1,13,23	l,v	e,n,z_{15}
S. II	13,23	l,z_{28}	1,5
S. II	13,23	l,z_{28}	z_6
S. II *vredelust*	1,13,23	l,z_{28}	z_{42}
S. adjame	13,23	r	1,6
S. linton	13,23	r	e,n,z_{15}
S. yarrabah	13,23	y	1,7
S. ordonez	1,13,23	y	l,w
S. tunis	1,13,23	y	z_6
S. II *nachshonim*	1,13,23	z	1,5
S. farmsen	13,23	z	1,6
S. worthington	1,13,23	z	l,w
S. ajiobo	13,23	z_4,z_{23}	–
S. III *arizonae* (Ar. 18:1,6,7:–)	13,23	z_4,z_{23},z_{32}	–
S. romanby	13,23	z_4,z_{24}	–
S. III *arizonae* (Ar. 18:1,3,11:–)	1,13,23	z_4,z_{24}	–
S. demerara	13,23	z_{10}	l,w
S. II	1,13,23	z_{29}	e,n,x
S. cubana[x]	1,13,23	z_{29}	–
S. anna	13,23	z_{35}	e,n,z_{15}
S. fanti	13,23	z_{38}	–
S. II *stevenage*	1,13,23	[z_{42}]	1,[5],7
S. II	13,23	–	1,6
	Group 06,14 (H)		
S. garba	1,6,14,25	a	1,5
S. ferlac	1,6,14,25	a	e,n,x
S. banjul	1,6,14,25	a	e,n,z_{15}
S. ndjamena	1,6,14,25	b	1,2
S. tucson	[1],6,14,[25]	b	[1,7]
S. III *arizonae* (Ar. 7a,7c:43:28)	(6),14	b	e,n,x
S. blijdorp	1,6,14,25	c	1,5
S. kassberg	1,6,14,25	c	1,6
S. runby	1,6,14,25	c	e,n,x
S. minna	1,6,14,25	c	l,w
S. heves	6,14,24	d	1,5
S. finkenwerder	[1],6,14,[25]	d	1,5
S. midway	6,14,24	d	1,7
S. florida	[1],6,14,[25]	d	1,7
S. lindern	6,14,25	d	e,n,x
S. charity	1,6,14,25	d	e,n,x
S. teko	1,6,14,25	d	e,n,z_{15}
S. encino	1,6,14,25	d	l,z_{13},z_{28}
S. albuquerque	1,6,14,24	d	z_6
S. bahrenfeld	6,14,24	e,h	1,5
S. onderstepoort	1,6,14,[25]	e,h	1,5
S. magumeri	1,6,14,25	e,h	1,6
S. beaudesert	[1],6,14,[25]	e,h	1,7
S. warragul	1,6,14,25	g,m	–
S. caracas	[1],6,14,[25]	g,m,s	–
S. catanzaro	6,14	g,s,t	–
S. II *rooikrantz*	1,6,14	m,t	1,5
S. II *emmerich*	6,14	[m,t]	e,n,x
S. kaitaan	1,6,14,25	m,t	–
S. mampeza	1,6,14,25	i	1,5
S. buzu	1,6,14,25	i	1,7
S. schalkwijk	6,14,24	i	e,n,..
S. moussoro	1,6,14,25	i	e,n,z_{15}
S. harburg	1,6,14,25	k	1,5
S. II	6,14	k	[e,n,x]
S. III *arizonae* (Ar. 7a,7c:29:31)	(6),14	k	z
S. II	1,6,14	k	z_6:z_{42}

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. III *arizonae* (Ar. 7a,7c:29:25)	(6),14	k	z_{53}
S. boecker	[1],6,14,[25]	l,v	1,7
S. horsham	[1],6,14,[25]	l,v	e,n,x
S. III *arizonae* (Ar. 7a,7c:23:31)	(6),14	l,v	z
S. III *arizonae* (Ar. 7a,7c:23:21)	(6),14	l,v	z_{35}
S. aflao	1,6,14,25	l,z_{28}	e,n,x
S. III *arizonae* (Ar. 7a,7c:24:31)	(6),14	r	z
S. surat	[1],6,14,[25]	r,[i]	e,n,z_{15}
S. carrau	6,14[24]	y	1,7
S. madelia	1,6,14,25	y	1,7
S. fischerkietz	1,6,14,25	y	e,n,x
S. mornington	1,6,14,25	y	e,n,z_{15}
S. homosassa	1,6,14,25	z	1,5
S. soahanina	6,14,24	z	e,n,x
S. sundsvall	1,6,14,25	z	e,n,x
S. poano	1,6,14,25	z	1,z_{13},z_{28}
S. bousso	1,6,14,25	z_4,z_{23}	[e,n,z_{15}]
S. IV	6,14	z_4,z_{23}	–
S. chichiri	6,14,24	z_4,z_{24}	–
S. uzaramo	1,6,14,25	z_4,z_{24}	–
S. nessa	1,6,14,25	z_{10}	1,2
S. II *bornheim*	1,6,14,25	z_{10}	1,(2),7
S. II *simonstown*	1,6,14	z_{10}	1,5
S. III *arizonae* (Ar. 7a,7c:27:28)	(6),14	z_{10}	e,n,x,z_{15}
S. III *arizonae* (Ar. 7a,7c:27:[31]:[38])	(6),14	z_{10}	[z]:[z_{66}]
S. II *slangkop*	1,6,14	z_{10}	z_6:z_{42}
S. potosi	6,14	z_{36}	1,5
S. sara	1,6,14,25	z_{38}	[e,n,x]
S. II	1,6,14	z_{42}	1,6
S. III *arizonae* (Ar. 7a,7c...:26:21)	1,6,14,25	z_{52}	z_{35}
	Group 016 (I)		
S. hannover	16	a	1,2
S. brazil	16	a	1,5
S. amunigun	16	a	1,6
S. nyeko	16	a	1,7
S. togba	16	a	e,n,x
S. fischerhuette	16	a	e,n,z_{15}
S. heron	16	a	z_6
S. hull	16	b	1,2
S. wa	16	b	1,5
S. glasgow	16	b	1,6
S. hvittingfoss	16	b	e,n,x
S. II	16	b	e,n,x
S. sangera	16	b	e,n,z_{15}
S. malstatt	16	b	z_6
S. II	16	b	z_{39}
S. II	16	b	z_{42}
S. vancouver	16	c	1,5
S. gafsa	16	c	1,6
S. shamba	16	c	e,n,x
S. hithergreen	16	c	e,n,z_{15}
S. oldenburg	16	d	1,2
S. II	16	d	1,5
S. sherbrooke	16	d	1,6
S. gaminara	16	d	1,7
S. barranquilla	16	d	e,n,x
S. nottingham	16	d	e,n,z_{15}
S. caen	16	d	l,w
S. barmbek	16	d	z_6
S. malakal	16	e,h	1,2
S. saboya	16	e,h	1,5
S. rhydyfelin	16	e,h	e,n,x
S. weston	16	e,h	z_6
S. II *bellville*	16	e,n,x	1,(5),7
S. tees	16	f,g	–
S. adeoyo	16	g,m	–
S. nikolaifleet	16	g,m,s	–
S. II *mobeni*	16	g,[m],[s],t	e,n,x

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. II *merseyside*	16	g,t	[1,5]
S. II	16	m,t	e,n,x
S. II *rowbarton*	16	m,t	[z_{42}]
S. mpouto	16	m,t	–
S. amina	16	i	1,5
S. wisbech	16	i	1,7
S. frankfurt	16	i	e,n,z_{15}
S. pisa	16	i	l,w
S. abobo	16	i	z_6
S. III *arizonae* (Ar. 25:33:21)	16	i	z_{35}
S. szentes	16	k	1,2
S. nuatja	16	k	e,n,x
S. orientalis	16	k	e,n,z_{15}
S. III *arizonae* (Ar. 25:29:31)	16	k	z
S. III *arizonae* (Ar. 25:22:21)	16	(k)	z_{35}
S. III *arizonae* (Ar. 25:29:25)	16	k	z_{53}
S. III *arizonae* (Ar. 25:23:30)	16	l,v	1,5,7
S. shanghai	16	l,v	1,6
S. welikade	16	l,v	1,7
S. salford	16	l,v	e,n,x
S. burgas	16	l,v	e,n,z_{15}
S. III *arizonae* (Ar. 25:23:31:[41])	16	l,v	z:[z_{61}]
S. losangeles	16	l,v,	z_6
S. III *arizonae* (Ar. 25:23:21)	16	l,v	z_{35}
S. III *arizonae* (Ar. 25:23:25)	16	l,v	z_{53}
S. westeinde	16	l,w	1,6
S. lomnava	16	l,w	e,n,z_{15}
S. II *noordhoek*	16	l,w	z_6
S. mandera	16	l,z_{13}	e,n,z_{15}
S. enugu	16	l,[z_{13}],z_{28}	[1,5]
S. battle	16	l,z_{13},z_{28}	1,6
S. ablogame	16	l,z_{13},z_{28}	z_6
S. II *sarepta*	16	l,z_{28}	z_{42}
S. rovaniemi	16	r,i	1,5
S. annedal	16	r,i	e,n,x
S. zwickau	16	r,i	e,n,z_{15}
S. saphra	16	y	1,5
S. akuafo	16	y	1,6
S. kikoma	16	y	e,n,x
S. avignon	16	y	e,n,z_{15}
S. fortlamy	16	z	1,6
S. lingwala	16	z	1,7
S. II *louwbester*	16	z	e,n,x
S. brevik	16	z	e,n,z_{15}
S. II	16	z	z_{42}
S. kibi	16	z_4,z_{23}	–
S. II *haddon*	16	z_4,z_{23}	–
S. IV *ochsenzoll*	16	z_4,z_{23}	–
S. IV *chameleon*	16	z_4,z_{32}	–
S. II	16	z_6	1,6
S. III *arizonae* (Ar. 25:27:30)	16	z_{10}	1,5,7
S. lisboa	16	z_{10}	1,6
S. III *arizonae* (Ar. 25:27:28)	16	z_{10}	e,n,x,z_{15}
S. redlands	16	z_{10}	e,n,z_{15}
S. angouleme	16	z_{10}	z_6
S. saloniki	16	z_{29}	–
S. II *jacksonville*	16	z_{29}	–
S. dakota	16	z_{35}	e,n,z_{15}
S. naware	16	z_{38}	–
S. II *woodstock*	16	z_{42}	1,(5),7
S. II *elsiesrivier*	16	z_{42}	1,6
S. III *arizonae* (Ar. 25:26:21)	16	z_{52}	z_{35}
	Group 017 (J)		
S. bonames	17	a	1,2
S. jangwani	17	a	1,5
S. kinondoni	17	a	e,n,x
S. kirkee	17	b	1,2
S. II *hillbrow*	17	b	e,n,x,z_{15}
S. bignona	17	b	e,n,z_{15}
S. II	17	b	z_6
S. victoriaborg	17	c	1,6
S. II *woerden*	17	c	z_{39}
S. berlin	17	d	1,5
S. niamey	17	d	l,w
S. jubilee	17	e,h	1,2
S. II *verity*	17	e,n,x,z_{15}	1,6
S. II	17	e,n,x,z_{15}	1,7
S. II *bleadon*	17	(f),g,t	[e,n,x,z_{15}]
S. II	17	g,t	z_{39}
S. bama	17	m,t	–
S. II	17	m,t	–
S. ahanou	17	i	1,7
S. III *arizonae* (Ar. 12:33:21)	17	i	z_{35}
S. irenea	17	k	1,5
S. matadi	17	k	e,n,x
S. II	17	k	–
S. morotai	17	l,v	1,2
S. michigan	17	l,v	1,5
S. carmel	17	l,v	e,n,x
S. III *arizonae* (Ar. 12:23:28)	17	l,v	e,n,x,z_{15}
S. III *arizonae* (Ar. 12:23:21)	17	l,v	z_{35}
S. granlo	17	l,z_{28}	e,n,x
S. lode	17	r	1,2
S. III *arizonae* (Ar. 12:24:31)	17	r	z
S. II	17	y	–
S. gori	17	z	1,2
S. warengo	17	z	1,5
S. tchamba	17	z	e,n,z_{15}
S. II *constantia*	17	z	l,w:z_{42}
S. III *arizonae* (Ar. 12:1,2,5:–) (Ar.12:1,2,6:–)	17	z_4,z_{23}	–
S. III *arizonae* (Ar. 12:1,6,7,9:–)	17	z_4,z_{23},z_{32}	–
S. III *arizonae* (Ar. 12:1,3,11:–)	17	z_4,z_{24}	–
S. III *arizonae* (Ar. 12:1,6,7:–) (Ar.12:1,7,8:–)	17	z_4,z_{32}	–
S. djibouti	17	z_{10}	e,n,x
S. III *arizonae* (Ar. 12:27:28:[38])	17	z_{10}	e,n,x,z_{15}:[z_{66}]
S. III *arizonae* (Ar. 12:27:31)	17	z_{10}	z
S. kandla	17	z_{29}	–
S. III *arizonae* (Ar. 12:16,17,18:–)	17	z_{29}	–
S. III *arizonae* (Ar. 12:17,20:–)	17	z_{36}	–
	Group 018 (K)		
S. brazos	6,14,18	a	e,n,z_{15}
S. fluntern	6,14,18	b	1,5
S. rawash	6,14,18	c	e,n,x
S. groenekan	18	d	1,5
S. usumbura	18	d	1,7
S. pontypridd	18	g,m	–
S. III *arizonae* (Ar. 7a,7b:13,14:–)	18	g,z_{51}	–
S. II	18	m,t	1,5
S. langenhorn	18	m,t	–
S. memphis	18	k	1,5
S. III *arizonae* (Ar. 7a,7b:22:25)	18	(k)	z_{53}
S. III *arizonae* (Ar. 7a,7b:22:34)	18	(k)	z_{54}
S. III *arizonae* (Ar. 7a,7b:23:28)	18	l,v	e,n,x,z_{15}
S. orlando	18	l,v	e,n,z_{15}
S. III *arizonae* (Ar. 7a,7b:23:31)	18	l,v	z
S. toulon	18	l,w	e,n,z_{15}
S. III *arizonae* (Ar. 7a,7b:24:31)	18	r	z

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. II	18	y	e,n,x,z_{15}
S. cerro	6,14,18	z_4,z_{23}	[1,5]
S. aarhus	18	z_4,z_{23}	z_{64}
S. II	18	z_4,z_{23}	–
S. III *arizonae* (Ar. 7a,7b:1,2,5:–) (Ar.7a,7b:1,2,6:–)	18	z_4,z_{23}	–
S. blukwa	18	z_4,z_{24}	–
S. III *arizonae* (Ar. 7a,7b:1,7,8:–)	18	z_4,z_{32}	–
S. carnac	18	z_{10}	z_6
S. II *zeist*	18	z_{10}	z_6
S. II *beloha*	18	z_{36}	–
S. IV	18	z_{36},z_{38}	–
S. sinthia	18	z_{38}	–
S. cotia	18	–	1,6
	Group 021 (L)		
S. assen	21	a	[1,5]
S. ghana	21	b	1,6
S. minnesota	21	b	e,n,x
S. hydra	21	c	1,6
S. rhone	21	c	e,n,x
S. II	21	c	e,n,x
S. spartel	21	d	1,5
S. magwa	21	d	e,n,x
S. madison	21	d	z_6
S. good	21	f,g	e,n,x
S. III *arizonae* (Ar. 22:13,14:–)	21	g,z_{51}	–
S. diourbel	21	i	1,2
S. III *arizonae* (Ar. 22:33:30)	21	i	1,5,7
S. III *arizonae* (Ar. 22:33:28)	21	i	e,n,x,z_{15}
S. III *arizonae* (Ar. 22:29:28)	21	k	e,n,x,z_{15}
S. III *arizonae* (Ar. 22:29:31)	21	k	z
S. III *arizonae* (Ar. 22:23:31)	21	l,v	z
S. III *arizonae* (Ar. 22:23:40$_a$,40$_c$)	21	l,v	z_{57}
S. keve	21	l,w	–
S. ruiru	21	y	e,n,x
S. II	21	z	–
S. baguida	21	z_4,z_{23}	–
S. III *arizonae* (Ar. 22:1,2,6:–)	21	z_4,z_{23}	–
S. IV *soesterberg*	21	z_4,z_{23}	–
S. II *gwaai*	21	z_4,z_{24}	–
S. III *arizonae* (Ar. 22:1,3,11:–)	21	z_4,z_{24}	–
S. III *arizonae* (Ar. 22:27:28)	21	z_{10}	e,n,x,z_{15}
S. III *arizonae* (Ar. 22:27:31)	21	z_{10}	z
S. II *wandsbek*	21	z_{10}	z_6
S. III *arizonae* (Ar. 22:16,17,18:–)	21	z_{29}	–
S. gambaga	21	z_{35}	e,n,z_{15}
S. III *arizonae* (Ar. 22:32^{ms}:28)	21	z_{65}	e,n,x,z_{15}
	Group 028 (M)		
S. solna	28	a	1,5
S. dakar	28	a	1,6
S. bakau	28	a	1,7
S. seattle	28	a	e,n,x
S. honelis	28	a	e,n,z_{15}
S. moero	28	b	1,5
S. ashanti	28	b	1,6
S. bokanjac	28	b	1,7
S. langford	28	b	e,n,z_{15}
S. II *kaltenhausen*	28	b	z_6
S. hermannswerder	28	c	1,5
S. eberswalde	28	c	1,6
S. halle	28	c	1,7
S. dresden	28	c	e,n,x
S. wedding	28	c	e,n,z_{15}
S. techimani	28	c	z_6
S. amoutive	28	d	1,5
S. hatfield	28	d	1,6

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. mundonobo	28	d	1,7
S. mocamedes	28	d	e,n,x
S. patience	28	d	e,n,z_{15}
S. cullingworth	28	d	l,w
S. kpeme	28	e,h	1,7
S. II	28	e,n,x	1,7
S. friedrichsfelde	28	f,g	–
S. abadina	28	g,m	[e,n,z_{15}]
S. II *llandudno*	28	g,(m),[s],t	1,5
S. croft	28	g,m,s	–
S. II	28	g,m,t	e,n,x
S. II	28	g,s,t	e,n,x
S. ona	28	g,s,t	–
S. II	28	m,t	[e,n,x]
S. vinohrady	28	m,t	–
S. doorn	28	i	1,2
S. cotham	28	i	1,5
S. volkmarsdorf	28	i	1,6
S. dieuppeul	28	i	1,7
S. warnemuende	28	i	e,n,x
S. kuessel	28	i	e,n,z_{15}
S. guildford	28	k	1,2
S. ilala	28	k	1,5
S. adamstown	28	k	1,6
S. ikeja	28	k	1,7
S. taunton	28	k	e,n,x
S. ank	28	k	e,n,z_{15}
S. leoben	28	l,v	1,5
S. vitkin	28	l,v	e,n,x
S. nashua	28	l,v	e,n,z_{15}
S. ramsey	28	l,w	1,6
S. fajara	28	l,z_{28}	e,n,x
S. bassadji	28	r	1,6
S. kibusi	28	r	e,n,x
S. II *oevelgoenne*	28	r	e,n,z_{15}
S. chicago	28	r,[i]	1,5
S. banco	28	r,i	1,7
S. sanktgeorg	28	r,[i]	e,n,z_{15}
S. oskarshamn	28	y	1,2
S. nima	28	y	1,5
S. pomona	28	y	1,7
S. kitenge	28	y	e,n,x
S. telaviv	28	y	e,n,z_{15}
S. shomolu	28	y	l,w
S. selby	28	y	z_6
S. ezra	28	z	1,7
S. brisbane	28	z	e,n,z_{15}
S. II *ceres*	28	z	z_{39}
S. teltow	28	z_4,z_{23}	1,6
S. babelsberg	28	z_4,z_{23}	[e,n,z_{15}]
S. rogy	28	z_{10}	1,2
S. farakan	28	z_{10}	1,5
S. malaysia	28	z_{10}	1,7
S. umbilo	28	z_{10}	e,n,x^5
S. luckenwalde	28	z_{10}	e,n,z_{15}
S. moroto	28	z_{10}	l,w
S. III *arizonae* (Ar. 35:27:[40a,40c])	28	z_{10}	[z_{57}]
S. djermaia	28	z_{29}	–
S. babili	28	z_{35}	1,7
S. aderike	28	z_{38}	e,n,z_{15}
	Group 030 (N)		
S. overvecht	30	a	1,2
S. zehlendorf	30	a	1,5
S. guarapiranga	30	a	e,n,x
S. doulassame	30	a	e,n,z_{15}
S. II *odijk*	30	a	z_{39}
S. louga	30	b	1,2
S. aschersleben	30	b	1,5
S. urbana	30	b	e,n,x

Table 5.11 (*Continued*)

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. neudorf	30	b	e,n,z_{15}
S. II	30	b	z_6
S. zaire	30	c	1,7
S. morningside	30	c	e,n,z_{15}
S. II	30	c	z_{39}
S. messina	30	d	1,5
S. livulu	30	e,h	1,2
S. II *slatograd*	30	f,g,t	–
S. godesberg	30	g,m	–
S. II	30	g,m,s	e,n,x
S. giessen	30	g,m,s	–
S. sternschanze[bb]	30	g,s,t	–
S. wayne	30	g,z_{61}	–
S. landau	30	i	1,2
S. morehead	30	i	1,5
S. soerenga	30	i	l,w
S. hilversum	30	k	1,2
S. ramatgan	30	k	1,5
S. aqua	30	k	1,6
S. angoda	30	k	e,n,x
S. odozi	30	k	e,n,[x],z_{15}
S. II	30	k	e,n,x,z_{15}
S. ligeo	30	l,v	1,2
S. donna	30	l,v	1,5
S. morocco	30	l,z_{13},z_{28}	e,n,z_{15}
S. gege	30	r	1,5
S. matopeni	30	y	1,2
S. bietri	30	y	1,5
S. steinplatz	30	y	1,6
S. baguirimi	30	y	e,n,x
S. nijmegen	30	y	e,n,z_{15}
S. bodjonegoro	30	z_4,z_{24}	–
S. II	30	z_6	1,6
S. sada	30	z_{10}	1,2
S. kumasi	30	z_{10}	e,n,z_{15}
S. aragua	30	z_{29}	–
S. kokoli	30	z_{35}	1,6
S. wuiti	30	z_{35}	e,n,z_{15}
S. ago	30	z_{35}	–
S. II	30	z_{39}	1,7
	Group 035 (O)		
S. umhlatazana	35	a	e,n,z_{15}
S. tchad	35	b	–
S. yolo	35	c	–
S. dembe[cc]	35	d	l,w
S. gassi	35	e,h	z_6
S. adelaide[dd]	35	f,g	–
S. II	35	f,g,t	1,5
S. ealing	35	g,m,s	–
S. II	35	g,m,s,t	–
S. ebrie	35	g,m,t	–
S. anecho	35	g,s,t	–
S. II	35	g,t	z_{42}
S. agodi	35	g,t	–
S. III *arizonae* (Ar. 20:13,14:–)	35	g,z_{51}	–
S. monschaui	35	m,t	–
S. III *arizonae* (Ar. 20:33:28)	35	i	e,n,x,z_{15}
S. gambia	35	i	e,n,z_{15}
S. bandia	35	i	l,w
S. III *arizonae* (Ar. 20:33:31)	35	i	z
S. III *arizonae* (Ar. 20:33:21)	35	i	z_{35}
S. III *arizonae* (Ar. 20:29:31)	35	k	z
S. III *arizonae* (Ar. 20:22:31)	35	(k)	z
S. III *arizonae* (Ar. 20:22:21)	35	(k)	z_{35}
S. III *arizonae*[ee] (Ar. 20:29:25)	35	k	z_{53}
S. III *arizonae* (Ar. 20:23:30)	35	l,v	1,5,7
S. III *arizonae* (Ar. 20:23:21)	35	l,v	z_{35}
S. II	35	l,z_{28}	–
S. III *arizonae* (Ar. 20:24:28)	35	r	e,n,x,z_{15}
S. massakory	35	r	l,w
S. III *arizonae* (Ar. 20:24:21)	35	r	z_{35}
S. III *arizonae* (Ar. 20:24:41)	35	r	z_{61}
S. alachua[ff]	35	z_4,z_{23}	–
S. III *arizonae* (Ar. 20:1,2,6:–)	35	z_4,z_{23}	–
S. westphalia	35	z_4,z_{24}	–
S. III *arizonae* (Ar. 20:1,7,8:–)	35	z_4,z_{32}	–
S. camberene	35	z_{10}	1,5
S. enschede	35	z_{10}	l,w
S. ligna	35	z_{10}	z_6
S. III *arizonae* (Ar. 20:27:21)	35	z_{10}	z_{35}
S. II *utbremen*	35	z_{29}	e,n,x
S. widemarsh	35	z_{29}	–
S. III *arizonae* (Ar. 20:16,17,18:–)	35	z_{29}	–
S. III *arizonae* (Ar. 20:17,20:–)	35	z_{36}	–
S. haga	35	z_{38}	–
S. III *arizonae* (Ar. 20:26:30)	35	z_{52}	1,5,7
S. III *arizonae* (Ar. 20:26:28)	35	z_{52}	e,n,x,z_{15}
S. III *arizonae* (Ar. 20:26:31)	35	z_{52}	z
S. III *arizonae* (Ar. 20:26:21)	35	z_{52}	z_{35}
	Group 038 (P)		
S. II	38	b	1,2
S. rittersbach	38	b	e,n,z_{15}
S. sheffield	38	c	1,5
S. kidderminster	38	c	1,6
S. II *carletonville*	38	d	[1,5]
S. thiaroye	38	e,h	1,2
S. kasenyi	38	e,h	1,5
S. korovi	38	g,m,[s]	–
S. II *foulpointe*	38	g,t	–
S. III *arizonae* (Ar. 16:13,14:–)	38	g,z_{61}	–
S. IV	38	g,z_{61}	–
S. mgulani	38	i	1,2
S. lansing	38	i	1,5
S. III *arizonae* (Ar. 16:33:25)	38	i	z_{53}
S. echa	38	k	1,2
S. mango	38	k	1,5
S. inverness	38	k	1,6
S. njala	38	k	e,n,x
S. III *arizonae* (Ar. 16:29:31)	38	k	z
S. III *arizonae* (Ar. 16:29:25)	38	k	z_{53}
S. III *arizonae* (Ar. 16:22:30)	38	(k)	1,5,7
S. III *arizonae* (Ar. 16:22:31)	38	(k)	z
S. III *arizonae* (Ar. 16:22:21:[38])	38	(k)	z_{35}:[z_{56}]
S. III *arizonae* (Ar. 16:22:34)	38	(k)	z_{54}
S. III *arizonae* (Ar. 16:22:37)	38	(k)	z_{55}
S. alger	38	l,v	1,2
S. kimberley	38	l,v	1,5
S. roan	38	l,v	e,n,x
S. III *arizonae* (Ar. 16:23:31)	38	l,v	z
S. III *arizonae* (Ar. 16:23:21)	38	l,v	z_{35}
S. III *arizonae* (Ar. 16:23:25:[34])	38	l,v	z_{35}:[z_{54}]
S. lindi	38	r	1,5
S. III *arizonae* (Ar. 16:24:30)	38	r	1,5,7
S. emmastad	38	r	1,6
S. III *arizonae* (Ar. 16:24:31:[40_a,40_b])	38	r	z:[z_{57}]
S. III *arizonae* (Ar. 16:24:21)	38	r	z_{35}
S. freetown	38	y	1,5
S. colombo	38	y	1,6
S. perth	38	y	e,n,x
S. yoff	38	z_4,z_{23}	1,2
S. IV	38	z_4,z_{23}	–
S. bangkok	38	z_4,z_{24}	–
S. III *arizonae* (Ar. 16:27:31)	38	z_{10}	z
S. III *arizonae* (Ar. 16:27:25)	38	z_{10}	z_{53}
S. klouto	38	z_{38}	–
S. III *arizonae* (Ar. 16:39:25)	38	z_{47}	z_{53}

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. III *arizonae* (Ar. 16:26:21)	38	z_{52}	z_{35}
S. III *arizonae* (Ar. 16:26:25)	38	z_{52}	z_{53}
	Group 039 (Q)		
S. II	39	a	z_{39}
S. wandsworth	39	b	1,2
S. abidjan	39	b	l,w
S. II	39	c	e,n,x
S. logone	39	d	1,5
S. mara	39	e,h	1,5
S. hofit	39	i	1,5
S. champaign	39	k	1,5
S. kokomlemle	39	l,v	e,n,x
S. oerlikon	39	l,v	e,n,z_{15}
S. II *mondeor*	39	l,z_{28}	e,n,x
S. anfo	39	y	1,2
S. windermere	39		1,5
	Group 040 (R)		
S. shikomah	40	a	1,5
S. greiz	40	a	z_6
S. II	1,40	a	z_6
S. II *springs*	40	a	z_{39}
S. riogrande	40	b	1,5
S. saugus	40	b	1,7
S. johannesburg	1,40	b	e,n,x
S. duval	1,40	b	e,n,z_{15}
S. benguella	40	b	z_6
S. II	40	b	–
S. II *suarez*	1,40	c	e,n,x,z_{15}
S. II	1,40	c	z_{39}
S. driffield	1,40	d	1,5
S. II *ottershaw*	40	d	–
S. tilene	1,40	e,h	1,2
S. II	1,40	(f),g	e,n,x,z_{15}
S. bijlmer	1,40	g,m	–
S. II *boksburg*	40	g,m,s,t	e,n,x
S. II *alsterdorf*	1,40	g,m,t	1,5
S. II	1,40	g,t	1,5
S. II	1,40	g,t	e,n,x
S. II	1,40	g,t	z_{42}
S. III *arizonae* (Ar. 10a,10b:13,14:28)	40	g,z_{51}	e,n,x,z_{15}
S. IV *seminole*	1,40	g,z_{51}	–
S. II	40	m,t	z_{39}
S. II	1,40	m,t	z_{42}
S. IV	40	m,t	–
S. III *arizonae* (Ar. 10a,10b:33:30)	40	i	1,5,7
S. goulfey	1,40	k	1,5
S. allandale	1,40	k	1,6
S. hann	40	k	e,n,x
S. II *sunnydale*	1,40	k	e,n,x,z_{15}
S. III *arizonae* (Ar. 10a,10b:29:31:40a,40c)	40	k	z:z_{57}
S. III *arizonae* (Ar. 10a,10b:29:25)	40	k	z_{53}
S. millesi	1,40	l,v	1,2
S. III *arizonae* (Ar. 10a,10b,(10c):23:31)	40	l,v	z
S. III *arizonae* (Ar. 10a,10b:23:25)	40	l,v	z_{53}
S. overchurch	40	l,w	–
S. bukavu	1,40	l,z_{28}	1,5
S. santhiaba	40	l,z_{28}	1,6
S. II *bulawayo*	1,40	z	1,5
S. casamance	40	z	e,n,x
S. nowawes	40	z	z_6
S. II	1,40	z	z_6
S. II	40	z	z_{39}
S. III *arizonae* (Ar. 10a,10b:1,2,5:–) (Ar.10a,10b:1,2,6:–)	40	z_4,z_{23}	–
S. IV *sachsenwald*	1,40	z_4,z_{23}	–
S. II *degania*	40	z_4,z_{24}	z_{39}
S. III *arizonae* (Ar. 10a,10b:1,3,11:–)	40	z_4,z_{24}	–
S. IV	40	z_4,z_{24}	–
S. III *arizonae* (Ar.10a,10b:1,7,8:–)	40	z_4,z_{32}	–
S. IV	40	z_4,z_{32}	–
S. II	1,40	z_6	1,5
S. trotha	40	z_{10}	z_6
S. III *arizonae* (Ar. 10a,10b:27:21)	40	z_{10}	z_{35}
S. omifisan	40	z_{29}	–
S. III *arizonae* (Ar. 10a,10b:16,17,18:–)	40	z_{29}	–
S. II *fandran*	1,40	z_{35}	e,n,x,z_{15}
S. III *arizonae* (Ar. 10a,10b:17,20:–)	40	z_{36}	–
S. II *grunty*	1,40	z_{39}	1,6
S. karamoja	1,40	z_{41}	1,2
S. II	1,40	[z_{42}]	1,(5),7
	Group 041 (S)		
S. II	41	b	[1,5]
S. II	41	b	1,7
S. vietnam	41	b	[z_6]
S. III *arizonae* (Ar. 13:32a,32b:28)	41	c	e,n,x,z_{15}
S. II	41	c	z_6
S. egusi	41	d	[1,5]
S. II *hennepin*	41	d	z_6
S. II *lethe*	41	g,t	–
S. III *arizonae* (Ar. 13:13,14:–)	41	g,z_{51}	–
S. leatherhead	41	m,t	1,6
S. II	41	k	–
S. III *arizonae* (Ar. 13:22:21)	41	(k)	z_{35}
S. II	41	l,z_{13},z_{28}	e,n,x,z_{15}
S. lumbumbashi	41	r	1,5
S. II *dubrovnik*	41	z	1,5
S. waycross	41	z_4,z_{23}	–
S. III *arizonae* (Ar. 13:1,2,5:–) (Ar.13:1,2,6:–)	41	z_4,z_{23}	–
S. IV	41	z_4,z_{23}	–
S. III *arizonae* (Ar. 13:1,6,7:–)	41	z_4,z_{23},z_{32}	–
S. ipswich	41	z_4,z_{24}	[1,5]
S. III *arizonae* (Ar. 13:1,3,11:–)	41	z_4,z_{24}	–
S. III *arizonae* (Ar. 13:1,7,8:–)	41	z_4,z_{32}	–
S. II *negev*	41	z_{10}	1,2
S. leipzig	41	z_{10}	1,5
S. landala	41	z_{10}	1,6
S. inpraw	41	z_{10}	e,n,x
S. II *lurup*	41	z_{10}	[e,n,x,z_{15}]
S. II *lichtenberg*	41	z_{10}	z_6
S. lodz	41	z_{29}	–
S. III *arizonae* (Ar. 13:16,17,18:–)	41	z_{29}	–
S. III *arizonae* (Ar. 13:17,20:–)	41	z_{36}	–
S. effa	41	z_{38}	–
S. II	41	–	1,6
	Group 042 (T)		
S. faji	1,42	a	e,n,z_{15}
S. II *chinovum*	42	b	1,5
S. II *uphill*	42	b	e,n,x,z_{15}
S. tomegbe	1,42	b	e,n,z_{15}
S. egusitoo	1,42	b	z_6
S. antwerpen	1,42	c	e,n,z_{15}
S. kampala	1,42	c	z_6
S. II *fremantle*	42	(f),g,t	–
S. maricopa	1,42	g,z_{51}	1,5
S. III *arizonae* (Ar. 15:13,14:–)	42	g,z_{51}	–
S. II	42	m,t	[e,n,x,z_{15}]

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. waral	1,42	m,t	–
S. kaneshie	1,42	i	l,w
S. middlesbrough	1,42	i	z_6
S. haferbreite	42	k	1,6
S. III *arizonae* (Ar. 15:29:31)	42	k	z
S. gwale	1,42	k	z_6
S. III *arizonae* (Ar. 15:22:21)	42	(k)	z_{35}
S. III *arizonae* (Ar. 15:23:30)	42	l,v	1,5,7
S. II *portbech*	42	l,v	e,n,x,z_{15}
S. III *arizonae* (Ar. 15:23:28)	42	l,v	e,n,x,z_{15}
S. coogee	42	l,v	e,n,z_{15}
S. III *arizonae* (Ar. 15:23:31)	42	l,v	z
S. III *arizonae* (Ar. 15:23:25)	42	l,v	z_{53}
S. II	42	l,z_{13},z_{28}	z_6
S. II	42	l,z_{28}	–
S. sipane	1,42	r	e,n,z_{15}
S. brive	1,42	r	l,w
S. III *arizonae* (Ar. 15:24:31)	42	r	z
S. III *arizonae* (Ar. 15:24:25)	42	r	z_{53}
S. II *nairobi*	42	r	–
S. III *arizonae*[23] (Ar. 15:24:–)	42	r	–
S. harvestehude	1,42	y	z_6
S. II *detroit*	42	z	1,5
S. ursenbach	42	z	1,6
S. II *rand*	42	z	e,n,x,z_{15}
S. II *nuernberg*	42	z	z_6
S. gera	1,42	z_4,z_{23}	1,6
S. III *arizonae* (Ar. 15:1,2,5:–) (Ar.15:1,2,6:–)	42	z_4,z_{23}	–
S. toricada	1,42	z_4,z_{24}	–
S. III *arizonae* (Ar. 15:1,3,11:–)	42	z_4,z_{24}	–
S. II	42	z_6	1,6
S. II	42	z_{10}	e,n,x,z_{15}
S. III *arizonae* (Ar. 15:27:28)	42	z_{10}	e,n,x,z_{15}
S. III *arizonae* (Ar. 15:27:31)	42	z_{10}	z
S. loenga	1,42	z_{10}	z_6
S. II	42	z_{10}	z_6
S. III *arizonae* (Ar. 15:27:21)	42	z_{10}	z_{35}
S. III *arizonae* (Ar. 15:27:38)	42	z_{10}	z_{56}
S. djama	1,42	z_{29}	–
S. kahla	1,42	z_{35}	1,6
S. weslaco	42	z_{36}	–
S. IV	42	z_{36}	–
S. vogan	1,42	z_{38}	z_6
S. taset	1,42	z_{41}	–
S. III *arizonae* (Ar. 15:26:31)	42	z_{52}	z
S. II	42		1,6
	Group 043 (U)		
S. graz	43	a	1,2
S. berkeley	43	a	1,5
S. II	43	a	z_6
S. II *kommetje*	43	b	z_{42}
S. montreal	43	c	1,5
S. II	43	d	e,n,x,z_{15}
S. II	43	d	z_{39}
S. II	43	d	z_{42}
S. II	43	e,n,x,z_{15}	1,(5),7
S. II	43	e,n,x,z_{15}	1,6
S. milwaukee	43	f,g	–
S. II	43	f,g,t	1,5
S. II *mosselbay*	43	g,m,[s],t	[z_{42}]
S. veddel	43	g,t	–
S. IV	43	g,z_{51}	–
S. II	43	g,z_{62}	e,n,x
S. mbao	43	i	1,2
S. thetford	43	k	1,2
S. ahuza	43	k	1,5
S. III *arizonae* (Ar.21:29:31)	43	k	z
S. III *arizonae* (Ar. 21:23:25)	43	l,v	z_{53}
S. III *arizonae* (Ar. 21:23:38)	43	l,v	z_{56}
S. III *arizonae* (Ar. 21:24:28)	43	r	e,n,x,z_{15}
S. III *arizonae* (Ar. 21:24:31)	43	r	z
S. III *arizonae* (Ar. 21:24:25)	43	r	z_{53}
S. farcha	43	y	1,2
S. kingabwa	43	y	1,5
S. ogbete	43	z	1,5
S. II	43	z	1,5
S. III *arizonae* (Ar. 21:1,2,5:–)	43	z_4,z_{23}	–
S. IV *houten*	43	z_4,z_{23}	–
S. III *arizonae* (Ar. 21:1,3,11:–)	43	z_4,z_{24}	–
S. IV	43	z_4,z_{24}	–
S. IV *tuindorp*	43	z_4,z_{32}	–
S. adana	43	z_{10}	1,5
S. II	43	z_{29}	e,n,x
S. II	43	z_{29}	z_{42}
S. IV	43	z_{29}	–
S. ahepe	43	z_{35}	1,6
S. III *arizonae* (Ar. 21:17,20:–)	43	z_{36}	–
S. IV *volksdorf*	43	z_{36},z_{38}	–
S. irigny	43	z_{38}	–
S. II *bunnik*	43	z_{42}	[1,5,7]
S. III *arizonae* (Ar. 21:26:25)	43	z_{52}	z_{53}
	Group 044 (V)		
S. niakhar	44	a	1,5
S. tiergarten	44	a	e,n,x
S. niarembe	44	a	l,w
S. sedgwick	44	b	e,n,z_{15}
S. madigan	44	c	1,5
S. quebec	44	c	e,n,z_{15}
S. bobo	44	d	1,5
S. kermel	44	d	e,n,x
S. fischerstrasse	44	d	e,n,z_{15}
S. II	1,44	e,n,x	1,6
S. vleuten	44	f,g	–
S. gamaba	44	g,m,s	–
S. II	44	g,t	z_{42}
S. carswell	44	g,z_{51}	–
S. IV	44	g,z_{51}	–
S. muguga	44	m,t	–
S. lawra	44	k	e,n,z_{15}
S. malika	44	l,z_{28}	1,5
S. brefet	44	r	e,n,z_{15}
S. V *camdeni*	44	r	–
S. uhlenhorst	44	z	l,w
S. kua	44	z_4,z_{23}	–
S. II	44	z_4,z_{23}	–
S. III *arizonae* (Ar. 1,3:1,2,5:–) (Ar.1,3:1,2,6:–)	44	z_4,z_{23}	–
S. IV	44	z_4,z_{23}	–
S. III *arizonae* (Ar. 1,3:1,6,7,9:–) (Ar. 1,3:1,2,10:–)	44	z_4,z_{23},z_{32}	–
S. christiansborg	44	z_4,z_{24}	–
S. III *arizonae* (Ar.1,3:1,3,11:-)	44	z_4,z_{24}	–
S. IV	44	z_4,z_{24}	–
S. III *arizonae* (Ar. 1,3:1,7,8:–)	44	z_4,z_{32}	–
S. IV *lohbruegge*	44	z_4,z_{32}	–
S. guinea	44	z_{10}	[1,7]
S. IV	44	z_{36},[z_{38}]	–
S. koketime	44	z_{38}	–
S. II *clovelly*	1,44	z_{39}	[e,n,x,z_{15}]
	Group 045 (W)		
S. II *vrindaban*	45	a	e,n,x
S. meekatharra	45	a	e,n,z_{15}
S. II *ejeda*	45	a	z_{10}
S. riverside	45	b	1,5
S. fomeco	45	b	e,n,z_{15}
S. deversoir	45	c	e,n,x
S. dugbe	45	d	1,6
S. karachi	45	d	e,n,x
S. suelldorf	45	f,g	–
S. tornow	45	g,m,[s]	–

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. II *windhoek*	45	g,m,s,t	1,5
S. II *bremen*	45	g,m,s,t	e,n,x
S. II *perinet*	45	g,m,t	e,n,x,z_{15}
S. binningen	45	g,s,t	–
S. III *arizonae* (Ar. 11:13,14:–)	45	g,z_{51}	–
S. IV	45	g,z_{51}	–
S. II	45	m,t	1,5
S. apapa	45	m,t	–
S. casablanca	45	k	1,7
S. cairns	45	k	e,n,z_{15}
S. II *klapmuts*	45	z	z_{39}
S. IV	45	z_4,z_{23}	–
S. III *arizonae* (Ar. 11:1,3,11:–)	45	z_4,z_{24}	–
S. III *arizonae* (Ar. 11:1,7,8:–)	45	z_4,z_{32}	–
S. II	45	z_{29}	1,5
S. II	45	z_{29}	z_{42}
S. jodhpur	45	z_{29}	–
S. III *arizonae* (Ar. 11:16,17,18:–)	45	z_{29}	–
S. lattenkamp	45	z_{35}	1,5
S. balcones	45	z_{36}	–
	Group 047 (X)		
S. II *bilthoven*	47	a	[1,5]
S. II	47	a	e,n,x,z_{15}
S. II *phoenix*	47	b	1,5
S. II *khami*	47	b	[e,n,x,z_{15}]
S. sya	47	b	z_6
S. saka	47	b	–
S. III *arizonae* (Ar. 28:43:–)	47	b	–
S. III *arizonae* (Ar. 28:32:30)	47	c	1,5,7
S. III *arizonae* (Ar.23:32:28) (Ar. 28:32:28:[40_a,40_c])	47	c	e,n,x,z_{15}:[z_{57}]
S. III *arizonae* (Ar. 28:32:31)	47	c	z
S. III *arizonae* (Ar. 28:32:21)	47	c	z_{35}
S. kodjovi	47	c	–
S. stellingen	47	d	e,n,x
S. II *quimbamba*	47	d	z_{39}
S. sljeme	1,47	f,g	–
S. luke	1,47	g,m	–
S. anie	47	(g),m,t	–
S. II	47	g,t	e,n,x
S mesbit	47	m,t	e,n,z_{15}
S. III *arizonae*[hh] (Ar. 23:33:28)	47	i	e,n,x,z_{15}
S. bergen	47	i	e,n,z_{15}
S. III *arizonae* (Ar. 28:33:31)	47	i	z
S. III *arizonae* (Ar. 23:33:21) (Ar.28:33:21)	47	i	z_{35}
S. III *arizonae* (Ar. 23:33:25) (Ar.28:33:25:[40_a,40_c])	47	i	z_{53}:[z_{57}]
S. staoueli	47	k	1,2
S. bootle	47	k	1,5
S. III *arizonae* (Ar. 28:29:30)	47	k	1,5,7
S. dahomey[ii]	47	k	1,6
S. III *arizonae* (Ar. 28:29:28)	47	k	e,n,x,z_{15}
S. lyon	47	k	e,n,z_{15}
S. III *arizonae* (Ar. 28:29:31)	47	k	z
S. III *arizonae* (Ar. 23:29:21)	47	k	z_{35}
S. III *arizonae* (Ar. 23:29:25)	47	k	z_{53}
S. III *arizonae*[ii] (Ar. 23:23:30)	47	l,v	1,5,(7)
S. III *arizonae* (Ar. 28:23:28)	47	l,v	e,n,x,z_{15}
S. III *arizonae* (Ar. 28:23:21)	47	l,v	z_{35}
S. III *arizonae* (Ar. 28:23:25)	47	l,v	z_{53}
S. III *arizonae* (Ar. 28:23:40a,40c)	47	l,v	z_{57}
S. teshie	1,47	l,z_{13},z_{28}	e,n,z_{15}
S. dapango	47	r	1,2
S. III *arizonae* (Ar. 23:24:30)	47	r	1,5,7
S. III *arizonae* (Ar. 23:24:31)	47	r	z
S. III *arizonae* (Ar. 23:24:21)	47	r	z_{35}
S. III *arizonae* (Ar. 23:24:25:[44])	47	r	z_{53}:[z_{60}]
S. III *arizonae*[kk] (Ar. 23:24:–)	47	r	
S. moualine	47	y	1,6
S. blitta	47	y	e,n,x
S. mountpleasant	47	z	1,5
S. kaolack	47	z	1,6
S. II	47	z	e,n,x,z_{15}
S. II *chersina*	47	z	z_6
S. tabligbo	47	z_4,z_{23}	e,n,z_{15}
S. bere[ii]	47	z_4,z_{23}	z_6
S. tamberma	47	z_4,z_{24}	–
S. II	47	z_6	1,6
S. III *arizonae* (Ar. 28:27:30)	47	z_{10}	1,5,7
S. III *arizonae* (Ar. 28:27:31)	47	z_{10}	z
S. III *arizonae* (Ar. 28:27:21)	47	z_{10}	z_{35}
S. ekpoti	47	z_{29}	–
S. III *arizonae* (Ar. 28:16,17,18:–)	47	z_{29}	–
S. bingerville	47	z_{35}	e,n,z_{15}
S. alexanderplatz	47	z_{38}	–
S. quinhon	47	z_{44}	–
S. III *arizonae* (Ar. 28:26:30)	47	z_{52}	1,5,7
S. III *arizonae* (Ar. 28:26:28)	47	z_{52}	e,n,x,z_{15}
S. III *arizonae* (Ar. 28:26:31)	47	z_{52}	z
S. III *arizonae* (Ar. 28:26:21)	47	z_{52}	z_{35}
	Group 048 (Y)		
S. hisingen	48	a	1,5,7
S. II	48	a	z_6
S. III *arizonae* (Ar. 5:35:[21])	48	a	[z_{35}]
S. II	48	a	z_{39}
S. II	48	b	z_6
S. III *arizonae* (Ar. 5,29:32:31)	48	c	z
S. II *hagenbeck*	48	d	z_6
S. fitzroy	48	e,h	1,5
S. II *hammonia*	48	e,n,x,z_{15}	z_6
S. II *erlangen*	48	g,m,t	–
S. III *arizonae* (Ar. 5:13,14:–)	48	g,z_{51}	–
S. IV *marina*	48	g,z_{51}	–
S. III *arizonae* (Ar. 5,29:33:31)	48	i	z
S. III *arizonae* (Ar. 29:33:21:[40])	48	i	z_{35}:[z_{57}]
S. III *arizonae* (Ar. 5:33:25)	48	i	z_{53}
S. III *arizonae* (Ar. 5:29:30)	48	k	1,5,(7)
S. II	48	k	e,n,x,z_{15}
S. III *arizonae* (Ar. 5:29:28)	48	k	e,n,x,z_{15}
S. dahlem	48	k	e,n,z_{15}
S. III *arizonae* (Ar. 5,29:31)	48	k	z
S. III *arizonae* (Ar.5:29:21)	48	k	z_{35}
S. II *sakaraha*	48	k	z_{39}
S. III *arizonae* (Ar. 5,29:29:25)	48	k	z_{53}
S. III *arizonae* (Ar. 5:22:25)	48	(k)	z_{53}
S. III *arizonae*[mm] (Ar. 5:23:30)	48	l,v	1,5,(7)
S. III *arizonae* (Ar. 5,29:23:31)	48	l,v	z
S. III *arizonae* (Ar. 5:24:28)	48	r	e,n,x,z_{15}
S. III *arizonae* (Ar. 5,29:24:31)	48	r	z
S. toucra[nn]	48	z	1,5
S. III *arizonae* (Ar. 5:1,2,5:–) (Ar.5:1,2,5,6:–) (Ar.5:1,6:–)	48	z_4,z_{23}	–
S. III *arizonae* (Ar. 5:1,6,7:–)	48	z_4,z_{23},z_{32}	–
S. djakarta	48	z_4,z_{24}	–
S. III *arizonae* (Ar. 5:1,3,11:–)	48	z_4,z_{24}	–
S. III *arizonae* (Ar. 5:1,7,8:–)	48	z_4,z_{32}	–
S. IV	48	z_4,z_{32}	–
S. II *ngozi*	48	z_{10}	[1,5]
S. isaszeg	48	z_{10}	e,n,x
S. III *arizonae* (Ar. 5:27:28)	48	z_{10}	e,n,x,z_{15}
S. III *arizonae* (Ar. 5,29:27:31)	48	z_{10}	z
S. II	48	z_{29}	–

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. V *bongor*	48	z_{35}	–
S. III *arizonae* (Ar. 5,29:17,20:–)	48	z_{36}	–
S. IV	48	z_{36},z_{38}	–
S. V *balboa*	48	z_{41}	–
S. III *arizonae* (Ar. 5,29:26:28)	48	z_{52}	e,n,x,z_{15}
S. III *arizonae* (Ar. 5:26:31)	48	z_{52}	z
	Group 050 (Z)		
S. rochdale	50	b	e,n,x
S. II	50	b	z_6
S. II *krugersdorp*	50	e,n,x	1,7
S. II *namib*	50	g,m,s,t	1,5
S. IV *wassenaar*	50	g,z_{51}	–
S. II *atra*	50	m,t	z_6:z_{42}
S. III *arizonae* (Ar. 9a,9c:33:30)	50	i	1,5,7
S. III *arizonae* (Ar. 9a,9c:33:28)	50	i	e,n,x,z_{15}
S. III *arizonae* (Ar. 9a,9c:33:31)	50	i	z
S. III *arizonae* (Ar. 9a,9c:29:30)	50	k	1,5,7
S. III *arizonae* (Ar. 9a,9c:29:28)	50	k	e,n,x,z_{15}
S. III *arizonae*[oo] (Ar. 9a,9b:29:31) (Ar. 9a,9c:29:31)	50	k	z
S. III *arizonae* (Ar. 9a,9b:22:31)	50	(k)	z
S. II *seaforth*	50	k	z_6
S. III *arizonae* (Ar. 9a,9b:29:21)	50	k	z_{35}
S. III *arizonae* (Ar. 9a,9b:22:21)	50	(k)	z_{35}
S. III *arizonae* (Ar. 9a,9c:29:25)	50	k	z_{53}
S. fass	50	l,v	1,2
S. III *arizonae* (Ar. 9a,9b:23:28)	50	l,v	e,n,x,z_{15}
S. III *arizonae* (Ar. 9a,9c:23:31)	50	l,v	z
S. III *arizonae* (Ar. 9a,9c:23:21)	50	l,v	z_{35}
S. II	50	l,w	e,n,x,z_{15}:z_{42}
S. II	50	l,z_{28}	z_{42}
S. III *arizonae* (Ar. 9a,9b:24:30)	50	r	1,5,(7)
S. III *arizonae* (Ar. 9a,9c:24:28)	50	r	e,n,x,z_{15}
S. III *arizonae* (Ar.9a,9b:24:31) (Ar. 9a,9c:24:31)	50	r	z
S. III *arizonae* (Ar. 9a,9b:24:21)	50	r	z_{35}
S. III *arizonae* (Ar. 9a,9b:24:25)	50	r	z_{53}
S. dougi	50	y	1,6
S. II *greenside*	50	z	e,n,x
S. III *arizonae* (Ar. 9a,9b:1,2,5:–) (Ar.9a,9b:1,2,6:–)	50	z_4,z_{23}	–
S. IV *flint*	50	z_4,z_{23}	–
S. III *arizonae* (Ar. 9a,9b:1,6,7:–)	50	z_4,z_{23},z_{32}	–
S. III *arizonae* (Ar. 9a,9b:1,3,11:–)	50	z_4,z_{24}	–
S. IV	50	z_4,z_{24}	–
S. III *arizonae* (Ar. 9a,9b:1,2,10:–) (Ar. 9a,9b:1,7,8:–)	50		–
S. IV *bonaire*	50	z_4,z_{32}	–
S. III *arizonae* (Ar. 9a,9c:27:31:[38])	50	z_{10}	z:[z_{66}]
S. II *hooggraven*	50	z_{10}	z_6:z_{42}
S. III *arizonae* (Ar. 9a,9c:27:25)	50	z_{10}	z_{53}
S. III *arizonae* (Ar. 9a,9b:16,17,18:–)	50	z_{29}	–
S. III *arizonae* (Ar. 9a,9b:17,20:–)	50	z_{36}	–
S. II *faure*	50	z_{42}	1,7
S. III *arizonae* (Ar. 9a,9b:26:30) (Ar. 9a,9c:26:30)	50	z_{42}	1,7
S. III *arizonae* (Ar. 9a,9b:26:31) (Ar. 9a,9c:26:31)	50	z_{52}	1,5,7
S. III *arizonae* (Ar. 9a,9b:26:21) (Ar. 9a,9c:26:21)	50	z_{52}	z
S. III *arizonae* (Ar. 9a,9b:26:25) (Ar. 9a,9c:26:25)	50	z_{52}	z_{35}
	Group 051		
S. tione	51	a	e,n,x
S. II	51	c	–
S. gokul	1,51	d	[1,5]
S. meskin	51	e,h	1,2
S. III *arizonae* (Ar. 1,2:13,14:–)	51	g,z_{51}	–
S. kabete	51	i	1,5
S. dan	51	k	e,n,z_{15}
S. III *arizonae* (Ar. 1,2:29:21)	51	k	z_{35}
S. overschie	51	l,v	1,5
S. dadzie	51	l,v	e,n,x
S. III *arizonae* (Ar. 1,2:23:31)	51	l,v	z
S. II *askraal*	51	l,z_{28}	[z_6]
S. antsalova	51	z	1,5
S. treforest	1,51	z	1,6
S. lechler	51	z	e,n,z_{15}
S. III *arizonae* (Ar. 1,2:1,2,5:–) (Ar. 1,2:1,2,6:–)	51	z_4,z_{23}	–
S. IV *harmelen*	51	z_4,z_{23}	–
S. III *arizonae* (Ar. 1,2:1,3,11:–)	51	z_4,z_{24}	–
S. II	51	z_{29}	e,n,x,z_{15}
S. II *roggeveld*	51	–	1,7
	Group 052		
S. uithof	52	a	1,5
S. ord	52	a	e,n,z_{15}
S. molesey	52	b	1,5
S. flottbek	52	b	[e,n,x]
S. II	52	c	k
S. utrecht	52	d	1,5
S. II	52	d	e,n,x,z_{15}
S. butare	52	e,h	1,6
S. derkle	52	e,h	1,7
S. saintemarie	52	g,t	–
S. II	52	g,t	–
S. III *arizonae* (Ar.31:29:21)	52	k	z_{35}
S. III *arizonae* (Ar. 31:29:25)	52	k	z_{53}
S. III *arizonae* (Ar. 31:23:25)	52	l,v	z_{53}
S. II *lobatsi*	52	z_{44}	1,5,7
S. III *arizonae* (Ar. 31:26:31)	52	z_{52}	z
	Group 053		
S. II	53	d	1,5
S. II	1,53	d	z_{39}
S. II	53	d	z_{42}
S. III *arizonae* (Ar. 1,4:13,14:–)	53	g,z_{51}	–
S. IV	1,53	g,z_{51}	–
S. III *arizonae* (Ar. 1,4:33:31)	53	i	z
S. III *arizonae* (Ar. 1,4:29:28)	53	k	e,n,x,z_{15}
S. III *arizonae* (Ar. 1,4:29:31)	53	k	z
S. III *arizonae* (Ar. 1,4:22:31)	53	(k)	z
S. III *arizonae* (Ar.1,4:22:21)	53	(k)	z_{35}
S. III *arizonae* (Ar. 1,4:23:28)	53	l,v	e,n,x,z_{15}
S. III *arizonae* (Ar. 1,4:23:21)	53	l,v	z_{35}
S. II *midhurst*	53	l,z_{28}	z_{39}
S. III *arizonae* (Ar. 1,4:24:31)	53	r	z

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. III *arizonae* (Ar. 1,4:24:21)	53	r	z_{35}
S. III *arizonae* (Ar. 1,4:24:38)	53	r	z_{56}
S. II	53	z	1,5
S. III *arizonae* (Ar. 1,4:31:30)	53	z	1,5,(7)
S. II	53	z	z_6
S. III *arizonae* (Ar. 1,4:1,2,5:–) (Ar.1,4:1,2,6:–)	53	z_4,z_{23}	–
S. IV	53	z_4,z_{23}	–
S. III *arizonae* (Ar.1,4:1,6,7:–) (Ar.1,4:1,6,7,9:–)	53	z_4,z_{23},z_{32}	–
S. II *humber*	53	z_4,z_{24}	–
S. III *arizonae* (Ar.1,4:1,3,11:–)	53	z_4,z_{24}	–
S. III *arizonae* (Ar. 1,4:27:21)	53	z_{10}	z_{35}
S. III *arizonae* (Ar. 1,4:16,17,18:–)	53	z_{29}	–
S. IV *bockenheim*	1,53	z_{36},z_{38}	–
S. III *arizonae* (Ar. 1,4:26:21)	53	z_{52}	z_{35}
S. III *arizonae* (Ar. 1,4:26:25)	53	z_{52}	z_{53}
	Group 054[pp]		
S. tonev	21,54	b	e,n,x
S. winnipeg	54	e,h	1,5
S. rossleben	54	e,h	1,6
S. borreze	54	f,g,s	–
S. uccle	3,54	g,s,t	–
S. poeseldorf	8,20,54	i	z_6
S. ochsenwerder	6,7,54	k	1,5
S. czernyring	54	r	1,5
S. steinwerder	3,15,54	y	1,5
S. yerba	54	z_4,z_{23}	–
S. canton	54	z_{10}	e,n,x
	Group 055		
S. II *tranoroa*	55	k	z_{39}
	Group 056		
S. II *artis*	56	b	–
S. II	56	d	–
S. II	56	e,n,x	1,7
S. II	56	l,z_{28}	–
S. III *arizonae* (Ar. 14:1.2.5:–) (Ar. 14:1,2,6:–)	56	z_4,z_{23}	–
S. III *arizonae* (Ar. 14:1,6,7,9:–)	56	z_4,z_{23},z_{32}	–
S. II	56	z_{10}	e,n,x
S. III *arizonae* (Ar. 1,14:16,18:–)	56	z_{29}	–
	Group 057		
S. antonio	57	a	z_6
S. maryland	57	b	1,7
S. III *arizonae* (Ar. 34:32:31:44)	57	c	z:z_{60}
S. II	57	d	1,5
S. II	57	g,m,s,t	z_{42}
S. II	57	g,t	–
S. III *arizonae* (Ar. 34:33:28)	57	i	e,n,x,z_{15}
S. III *arizonae* (Ar. 34:33:31)	57	i	z
S. IV	57	z_4,z_{23}	–
S. II *locarno*	57	z_{29}	z_{42}
S. II *manombo*	57	z_{39}	e,n,x,z_{15}
S. II *tokai*	57	z_{42}	1,6:z_{63}
	Group 058		
S. II	58	a	[z_6]
S. II	58	b	1,5
S. II	58	c	z_6
S. II	58	d	z_6
S. III *arizonae* (Ar. 1,33:33:28)	58	i	e,n,x,z_{15}
S. III *arizonae* (Ar. 1,33:23:28)	58	l,v	e,n,x,z_{15}
S. III *arizonae* (Ar. 1,33:23:21)	58	l,v	z_{35}
S. II *basel*	58	l,z_{13},z_{28}	1,5
S. III *arizonae* (Ar. 1,33:24:28)	58	r	e,n,x,z_{15}
S. III *arizonae* (Ar. 1,33:24:31)	58	r	z
S. III *arizonae*[qq] (Ar. 1,33:24:25[40a,40c])	58	r	z_{53}:[z_{57}]
S. II	58	z_{10}	1,6
S. II	58	z_{10}	z_6
S. III *arizonae* (Ar. 1,33:26:31)	58	z_{52}	z
S. III *arizonae* (Ar. 1,33:26:21)	58	z_{52}	z_{35}
	Group 059		
S. III *arizonae* (Ar. 19:32:28)	59	c	e,n,x,z_{55}
S. III *arizonae* (Ar. 19:33:31)	59	i	z
S. III *arizonae* (Ar. 19:33:21)	59	i	z_{35}
S. III *arizonae* (Ar. 19:22:28)	59	(k)	e,n,x,z_{15}
S. II *betioky*	59	k	(z)
S. III *arizonae* (Ar. 19:22:31)	59	(k)	z
S. III *arizonae* (Ar. 19:22:21)	59	(k)	z_{35}
S. III *arizonae* (Ar. 19:29:25)	59	k	z_{53}
S. III *arizonae* (Ar. 19:23:31)	59	l,v	z
S. III *arizonae* (Ar. 19:23:25)	59	l,v	z_{53}
S. III *arizonae* (Ar.19:1,2,5:–) (Ar. 19:1,2,6:–)	59	z_4,z_{23}	
S. III *arizonae* (Ar. 19:27:25)	59	z_{10}	z_{53}
S. III *arizonae* (Ar.19:27:40$_a$,40$_c$)	59	z_{10}	z_{57}
S. III *arizonae* (Ar. 19:16,17,18:–)	59	z_{29}	–
S. III *arizonae* (Ar. 19:17,20:–)	59	z_{36}	–
S. III *arizonae* (Ar. 19:26:–)	59	z_{52}	–
	Group 060		
S. II *setubal*	60	g,m,t	z_6
S. III *arizonae* (Ar. 24:33:28)	60	i	e,n,x,z_{15}
S. III *arizonae* (Ar. 24:33:21	60	i	z_{35}
S. III *arizonae* (Ar.24:29:31)	60	k	z
S. III *arizonae* (Ar. 24:29:21)	60	k	z_{35}
S. III *arizonae* (Ar. 24:22:25)	60	(k)	z_{53}
S. III *arizonae* (Ar. 24:23:31)	60	l,v	z
S. III *arizonae* (Ar. 24:24:28)	60	r	e,n,x,z_{15}
S. III *arizonae* (Ar. 24:24:31)	60	r	z
S. III *arizonae* (Ar. 24:24:21)	60	r	z_{35}
S. III *arizonae* (Ar. 24:24:25)	60	r	z_{53}
S. II *luton*	60	z	e,n,x
S. III *arizonae* (Ar. 24:27:31)	60	z_{10}	z
S. III *arizonae* (Ar. 24:27:21)	60	z_{10}	z_{35}
S. III *arizonae* (Ar. 24:26:30)	60	z_{52}	1,5,7
S. III *arizonae* (Ar.24:26:31)	60	z_{52}	z
S. III *arizonae* (Ar.24:26:21)	60	z_{52}	z_{35}
S. III *arizonae* (Ar. 24:26:25)	60	z_{52}	z_{53}
	Group 061		
S. III *arizonae* (Ar. 26:32:30)	61	c	1,5,(7)
S. III *arizonae* (Ar. 26:32:21)	61	c	z_{35}
S. III *arizonae* (Ar. 26:33:28)	61	i	e,n,x,z_{15}
S. III *arizonae* (Ar. 26:33:31)	61	i	z
S. III *arizonae* (Ar. 26:33:21)	61	i	z_{35}
S. III *arizonae* (Ar. 26:33:25)	61	i	z_{53}
S. III *arizonae* (Ar. 26:29:30)	61	k	1,5,(7)
S. III *arizonae* (Ar. 26:22:25)	61	(k)	z_{53}
S. III *arizonae* Ar. 26:23:30:[40$_a$,40$_b$])	61	l,v	1,5,7:[z_{57}]
S. III *arizonae* (Ar. 26:23:31)	61	l,v	z
S. III *arizonae* (Ar. 26:23:21)	61	l,v	z_{35}
S. III *arizonae* (Ar. 26:24:30)	61	r	1,5,7
S. III *arizonae* (Ar. 26:24:21)	61	r	z_{35}
S. III *arizonae* (Ar. 26:24:25)	61	r	z_{53}
S. III *arizonae* (Ar. 26:27:21)	61	z_{10}	z_{35}
S. III *arizonae* (Ar. 26:26:30)	61	z_{52}	1,5,7
S. III *arizonae* (Ar. 26:26:31)	61	z_{52}	z
S. III *arizonae* (Ar. 26:26:21)	61	z_{52}	z_{35}
S. III *arizonae* (Ar. 26:26:25)	61	z_{52}	z_{53}
	Group 062		
S. III *arizonae* (Ar. 6:13,14:–)	62	g,z_{61}	–
S. III *arizonae* (Ar. 6:1,2,5:–)	62	z_4,z_{23}	–

Table 5.11—*continued*

Serovar	Somatic (O) antigens	Flagellar (H) Antigens	
		Phase 1	Phase 2
S. III *arizonae* (Ar. 6:1,7,8:–)	62	z_4,z_{32}	–
	Group 063		
S. III *arizonae* (Ar. 8:13,14:–)	63	g,z_{51}	–
S. III *arizonae* (Ar. 8:1,2,5)	63	z_4,z_{23}	–
S. III *arizonae* (Ar. 8:1,7,8:–)	63	z_4,z_{32}	–
S. III *arizonae* (Ar. 8:17,20:–)	63	z_{36}	–
	Group 065[r]		
S. III *arizonae* (Ar. 30:32:30)	65	c	1,5,7
S. III *arizonae* (Ar. 30:32:31)	65	c	z
S. III *arizonae* (Ar. 30:32:25)	65	c	z_{63}
S. II	65	(f),g,t	–
S. III *arizonae* (Ar. 30:33:28)	65	i	e,n,x,z_{15}
S. III *arizonae* (Ar. 30:22:31)	65	(k)	z
S. III *arizonae* (Ar. 30:22:21)	65	(k)	z_{35}
S. III *arizonae* (Ar. 30:22:25)	65	(k)	z_{63}
S. III *arizonae* (Ar. 30:23:28)	65	l,v	e,n,x,z_{15}
S. III *arizonae* (Ar. 30:23:31)	65	l,v	z
S. III *arizonae* (Ar. 30:23:21)	65	l,v	z_{35}
S. III *arizonae* (Ar. 30:23:25)	65	l,v	z_{53}
S. III *arizonae* (Ar. 30:27:28)	65	z_{10}	e,n,x,z_{15}
S. III *arizonae* (Ar. 30:27:31)	65	z_{10}	z
S. III *arizonae* (Ar. 30:26:31)	65	z_{62}	z
S. III *arizonae* (Ar. 30:26:21)	65	z_{62}	z_{35}
S. III *arizonae* (Ar. 30:26:25)	65	z_{62}	z_{63}
S. II	65		1,6
	Group 066		
S. V *maregrosso*	66	z_{35}	–
S. V *brookfield*	66	z_{41}	–
S. V *malawi*	66	z_{65}	–
	Group 067		
S. crossness	67	r	1,2

carrier state is not monitored by periodic stool cultures. Antibiotics that are active in curing the disease (e.g., chloramphenicol or thiophenicol for typhoid fever) are ineffective in the treatment of the carrier state.

Strains of *Salmonella* from urine are often of the R form. Bilharziosis has to be controlled in *Salmonella* carriers (LoVerde, 1980). Sickle-cell anemia must be suspected in cases of osteomyelitis due to *Salmonella* in black children (Vandepitte, 1953).

Antibiotic and drug sensitivity. Salmonella strains such as *E. coli* can readily acquire plasmids that contain genes that confer resistance to antibiotics. Multiple resistance is selected for when antibiotics are used extensively in hospitals or added to feed. The same plasmids may be found in strains of human or animal origin (Anderson et al., 1975). Serovars strictly adapted to man, such as *S. typhi*, may acquire resistance to chloramphenicol as the result of the long term, indiscriminate use of this drug or other antibiotics (Anderson, 1975).

Ecology. Although some *Salmonella* serovars are strictly host-adapted, the majority have a wide host range (e.g. *S. typhimurium*). Some are localized in a particular region of the globe (e.g. "*S. sendai*" in the Far East, "*S. berta*" in North America), but others are ubiquitous (e.g. *S. typhimurium*). Strains belonging to "subgenera" II and III are frequently isolated from the intestinal contents of cold-blooded animals and only rarely from warm-blooded animals. Strains of "subgenera" IV and V are isolated chiefly from the environment and are rarely pathogenic for man.

Isolation and Enrichment Procedures

Isolation from blood is done according to the classical method for hemoculture. A biphasic culture bottle containing a vertical agar layer along one side and a broth medium at the bottom (Castaneda, 1957; Hall et al., 1979; Krieg and Gerhardt, 1981) prepared with tryptic soy agar/broth containing 2% sodium citrate is convenient. Isolated colonies grow on the agar layer. Identification is usually done by (a) diagnosis of the family *Enterobacteriaceae*, (b) diagnosis of the genus *Salmonella* (diagnosis of the "subgenus" for strains isolated from blood cultures is not routinely necessary, because almost all blood isolates belong to "subgenus" I), (c) diagnosis of the serovar, (d) determination of the antibiotic susceptibility pattern, and (e) further study of the biovar and phagovar if indicated.

Selective procedures are needed for the isolation of *Salmonella* from specimens containing mixed bacterial flora (fecal samples, autopsy samples, food, environmental samples, etc. Enrichment (i.e. an increased ratio of *Salmonella* cells to other bacterial cells during incubation) is obtained using liquid nutrient media containing selective agents that inhibit or retard the growth of bacteria other than *Salmonella*. Use of enrichment media is essential when the number of salmonellae in a sample is very low, i.e., when the probability of finding colonies by direct isolation is low. Three media may be recommended for general use: (a) the tetrathionate medium of Muller (1923); (b) Muller's medium modified by Kauffman (1935) by addition of bile and brilliant green; and (c) selenite broth devised by Leifson (1936). Tetrathionate and selenite broth are suitable for all *Salmonella* serovars. Tetrathionate-bile-brillant green medium is suitable for all except host-adapted serovars such as *S. typhi*. Enrichment media should be heavily inoculated, e.g., 0.5 ml of fecal suspension per 10 ml of medium. After incubation for 18 h at 37°C, a loopful of enrichment culture is streaked onto agar plating medium.

The same enrichment media may be used for detection of salmonellae in water. The simplest method is to add one volume of the water sample to an equal volume of double-strength medium. For detecting salmonellae in food, a generally suitable procedure is to inoculate 25 g of the suspected food into 225 ml of selenite F broth, incubate for 24 h and isolated on selective agar media. In the case of a dehydrated food, nutrient broth containing the sample is incubated overnight before inoculation of enrichment media.

Agar media are used for isolation of salmonellae. Streaking a loopful of enrichment culture or a suspension of the sample (e.g., stool) should be done carefully in order to obtain the greatest number of perfectly isolated colonies. Because the most discriminating character is lactose fermentation, the majority of media for isolation contain lactose and a pH indicator. In addition, the media contain selective agents to inhibit the growth of non-*Salmonella* organisms and the swarming of *Proteus mirabilis* and *P. vulgaris*. Some media also contain ferrous citrate for the detection of H_2S-producing bacteria.

Examples of media of moderate selectivity are: (a) *MacConkey agar*, which contains lactose, neutral red, and the selective inhibitors crystal violet and bile salts. Lactose-positive colonies are red, lactose-negative colonies are colorless. (b) *desoxycholate citrate agar*, which contains lactose, neutral red, and the selective agent desoxycholate. Ferric ammonium citrate is included as an indicator of H_2S production. Lactose-positive colonies are red, lactose-negative colonies are colorless. If H_2S is produced, the inner part of the colony is black.

Examples of media of higher selectivity are: (a) *SS agar*, which contains lactose, neutral red, and the selective agents brilliant green and bile salts. Ferric citrate is an indicator of H_2S production. The appearance of colonies is the same as on desoxycholate citrate agar. (b) *brilliant green agar*, which contains lactose, phenol red, and the selective agent brilliant green. This medium is easy to prepare and is suitable for all salmonellae except host-adapted serovars. It is not suitable for shigellae. Lactose-positive colonies are green, lactose-negative colonies are pink. All of the above-mentioned media are reviewed in the books by Kauffmann (1966) and Edwards and Ewing (1972). (c) *Hektoen medium* (King and Metzer, 1968), which contains lactose, sucrose and salicin, a mixture of bromothymol blue and Andrade's pH indicator, ferric citrate to detect H_2S production, and sodium desoxycholate as a selective inhibitor. Colonies that do not ferment any of the three sugars (e.g. *Salmonella*) are blue-green, with a black center if H_2S is produced. Colonies fermenting one or more of the sugars (e.g., *Escherichia coli*, *Enterobacter cloacae*) are salmon-colored. This medium is suitable for all *Salmonella* serovars and for shigellae.

A general procedure for the detection of salmonellae in feces or food as follows. A suspension of the sample in saline is streaked onto the chosen isolation medium and also inoculated into an enrichment broth. After overnight incubation, the plating medium is examined for suspect colonies (lactose-negative, H_2S-positive or negative); also, a loopful of the enrichment culture is streaked onto another plate of selective agar medium. After overnight incubation, this plate is also examined for suspect colonies. A quick screening of several suspect colonies is done by inoculating each into a few drops of urea medium and incubating at 37°C for 2 h. Biochemical characterization is continued only for urease-negative colonies (urease-positive colonies growing at 18 h are likely to be *Proteus*). *Salmonella* must be differentiated mainly from *Citrobacter freundii*, *Proteus mirabilis*, *Hafnia alvei*, and, in food bacteriology, *Alteromonas putrefaciens*. To detect *Salmonella arizonae*, attention should be given to *lactose-positive*, H_2S-positive colonies on plating media.

Maintenance Procedures

Salmonella cultures remain viable for many years when stored on peptone agar) meat extract, 5.0 g; peptone, 10.0 g; NaCl, 3.0; $Na_2HPO_4 \cdot 12H_2O$, 2.0 g; agar, 10.0 g; distilled water, 1,000 ml; pH 7.4) distributed into small, tightly stoppered, screw-capped tubes. This medium is stab-inoculated and kept in the dark at room temperature. Lyophilization also gives good results. For lyophilization, it is necessary to isolate each subculture and to select a colony with the desired serologic characteristics.

Differentiation from other closely related genera

Characteristics useful for differentiating the genus *Salmonella* from other *Enterobacteriaceae* are given in Table 5.3 of the chapter on the family *Enterobacteriaceae*.

Taxonomic Comments

If one accepts the principle that bacteria which are related by 70% or more on the basis of DNA/DNA hybridization experiments belong to the same "genospecies," the so-called "genus" *Salmonella* is, in fact, one species (Crosa, 1973). In other words, all salmonellae and arizonae form one species composed of five subgroups: typical *Salmonella*, atypical *Salmonella* "subgenus" II, atypical *Salmonella* "subgenus" IV, monophasic "subgenus" III (*S. arizonae*), and diphasic "subgenus" III (*S. arizonae*) (Brenner, 1978; Stoleru et al., 1976). Genetically, the level of Kauffman's four "subgenera," including the discrimination between the monophasic and diphasic strains of "subgenus" III and the new "subgenus" V (Le Minor, unpublished results; see Table 5.10) is that of subspecies. "Nevertheless the schemes now in use will continue to be used because people are familiar with them and are very slow to adjust to a new system" (Brenner, 1978).

The names given to salmonellae do not follow the usual rules of nomenclature. Because of their importance in pathology, the first salmonellae were given names which indicated the disease and/or the animal from which the organism was isolated, and names of this kind (such as *S. typhi*, "*S. paratyphi-A*," *S. choleraesuis*, *S. typhimurium* and "*S. abortusovis*") continue to be used in clinical bacteriology. This nomenclature was abandoned by the more systematically minded, for these names implied that pathogenicity was limited to definite host species, whereas this is not generally true. For example, *S. typhimurium* and "*S. bovismorbificans*" are frequently isolated from human infections. New types are now given the name of the town, region or country in which the first strain was isolated, e.g., "*S. london*," "*S. panama*," "*S. stanleyville*," etc. New types of "subgenera" II, III and IV described since 1966 are designated simply by antigenic formula; this allows the "Arizona" group ("subgenus" III, or *S. arizonae*) of Edwards, Fife and Ramsey (1959) to be included in the Kauffmann-White scheme, simplifies the terminology of the antigenic factors, and allows the same antisera to be used to establish antigenic formulae (Kauffmann and Rohde, 1962; Kauffmann, 1965; Rohde, 1967). With few exceptions, the formulae of "*Arizona*" serovars published by Edwards, Fife and Ewing (1965) may be translated into *Salmonella* formulae and included in the Kauffmann-White scheme.

The International Subcommittee on *Enterobacteriaceae* has not given clear guidance on the naming of the differing serovars. It is paradoxical that serovars of "subgenus" I bear species-like epithets, while those of *Escherichia coli* and *Salmonella* "subgenus" III (i.e., *S. arizonae*) do not. *S. typhi* owes its name to the importance of the bacterium in human pathology, but when these infection syndrome names were first applied no one could have imagined that by 1981 there would be more than 2,000 closely related serovars. Borman, Stuart and Wheeler (1944) proposed the subdivision of the genus into three species, *S. choleraesuis* (the type species), "*S. typhosa*" (*S. typhi*) and "*S. kauffmannii*", the last to serve as a species for all the serological types. Kauffman and Edwards (1952) made a similar proposal, but designated the all-embracing species "*Salmonella enterica*." Ewing (1966) proposed a three-species concept, with *S. enteritidis* representing all serovars other than *S. typhi* and *S. choleraesuis*. Another proposal (Le Minor, Rohde and Taylor, 1970) was to consider Kauffmann's "subgenera" as species: "*S. kauffmannii*" ("subgenus" I), *S. salamae* ("subgenus" II), *S. arizonae* ("subgenus" III) and "*S. houtenae*" ("subgenus" IV). Serovars of "*S. kauffmannii*" would be designated by their species names followed by that of their serovar (e.g., "*S. kauffmannii*" serovar *typhi*), and serovars of "*S. salamae*," *S. arizonae* and "*S. houtenae*" would be designated by their species names followed by their antigenic formulae. Kauffmann (1971, 1973) disagreed with all of the preceding propositions and considered a species as a "group of related sero-fermentative phagetypes" in his "Realität Theorie" (reviewed 1978).

Scientifically, none of the present methods of nomenclature of salmonellae is satisfactory. Without prejudice as to what constitutes a species, the *Enterobacteriaceae* subcommittee considers the diagnostic use of the Kauffmann-White scheme to be overridingly important and that the practice of giving names to the serovars of "subgenus" I should continue, but that new serovars of the other subgenera should be designated only by their antigenic formulae.

Editorial Note

On the basis of numerical taxonomy and DNA relatedness studies, Le Minor, Véron and Popoff (Ann. Microbiol. (Inst. Pasteur) 133B: 245–254, 1982) recently proposed nomenclatural changes for salmonellae, as follows. The genus should consist of a single species, *S. choleraesuis*, having six subspecies: (a) the subspecies *choleraesuis*, corresponding to the former subgenus I; (b) the subspecies *salamae*, corresponding to the former subgenus II; (c) the subspecies *arizonae*, corresponding to the monophasic serovars of the former subgenus III; (d) the subspecies *diarizonae*, corresponding to the diphasic serovars of the former subgenus III; (e) the subspecies *houtenae*, corresponding to the former subgenus IV, and (f) the subspecies *bongori*, composed of strains that are positive for dulcitol, ONPG and KCN. Type strains were proposed for each subspecies.

Further Reading

Edwards, P.R. and W. H. Ewing. 1972. Identification of *Enterobacteriaceae*, 3rd Ed, Burgess Publishing, Minneapolis, Minn.
Kaufmann, F. 1966. The Bacteriology of *Enterobacteriaceae*, Munksgaard, Copenhagen.
Kauffmann, F. 1978. *Das Fundament*, Munksgaard, Copenhagen.
Kelterborn, E. 1967. Salmonella-Species, Hirzel, Leipzig.
Van Oye, E. 1964. *The World Problem of Salmonellosis*, Junk, The Hague.

Differentiation of the "subgenera" of the genus Salmonella

The biochemical characteristics which differentiate the five "subgenera" of the genus *Salmonella* are presented in Table 5.10.

Differentiation of the serovars of the genus **Salmonella**

The antigenic formulae of the salmonellae (i.e. the Kauffmann-White scheme) are given in Table 5.11. An alphabetical listing of *Salmonella* serovars, indicating their "subgenus" and O group, is presented in Table 5.12.

List of selected serovars of the genus **Salmonella**

"Subgenus" I

a. *Salmonella choleraesuis* (Smith 1894) Weldin 1927, 155.[AL] (*Bacillus cholerae suis* Smith 1894, 9.) *Editorial Note*: although the specific epithet *cholerae-suis* is listed in the Approved Lists of Bacterial Names (1980), the hyphen should not be used (J. J. Farmer III, Int. J. Syst. Bacteriol. *33:* 425, 1983).

chol.er.ae.su'is. Gr. n. *cholera* cholera; L. n. *sus* hog; M.L. gen. n. *suis* of a hog; M.L. gen. n. *choleraesuis* of hog cholera.

Antigenic formula: 6,7,c:1,5. The detailed O antigen formula is normally 6_2,7, but this may be transformed by lysogenization into 6_1,7 or 6_2,7,14.

Arabinose and trehalose are not fermented; dulcitol is slowly and irregularly fermented.

Those strains which produce H_2S are designated as *S. choleraesuis* biovar *kunzendorf.*

Pathogenic for man and other animals.

Type strain: ATCC 13313 (NCTC 5735).

b. "*Salmonella hirschfeldii*" Weldin 1927, 161. (Paratyphoid C bacillus, Hirschfeld 1919, 296; *Salmonella paratyphi-C* Salmonella Subcommittee 1934.)

hirsch.fel.di.i. M.L. gen. n. *hirschfeldii* of Hirschfeld; named after Hirschfeld, who first called the organism the paratyphoid C bacillus, a name still in common use today.

Antigenic formula; 6,7,[Vi]:c:1,5.

Ferments dulcitol and trehalose; produces H_2S. Arabinose fermentation is variable.

c. *Salmonella typhi* (Schroeter 1886) Warren and Scott 1930, 416.[AL] (*Bacillus typhi* Schroeter 1886, 165.)

ty'phi. Gr. n. *typhus* a stupor; M.L. gen. n. *typhi* of typhoid.

Antigenic formula: 9,12,[Vi]:d:–. Wild strains may possess H antigen z_{66} instead of H antigen d (Guinée et al., 1981).

Does not grow on Simmons' citrate medium or on a minimal defined medium; requires tryptophan as a growth factor.

Does not produce gas from glucose or other sugars. Fermentation of xylose is variable.

Many strains are agglutinated by anti-Vi serum and are inagglutinable by 09 serum; their colonies are opaque and have an iridescent appearance when examined by transmitted light. Colonies of intermediate appearance agglutinable by both Vi and O antisera, may occur (VW colonies).

Pathogenic only for man, causing typhoid (enteric) fever; transmitted by water or food contaminated by human excreta.

Type strain: ATCC 19430.

d. "*Salmonella paratyphi-A*" (Brion and Kayser 1902) Castellani and Chalmers 1919, 939. (*Bacterium paratyphi* Kayser 1902, 426; *Bacterium paratyphi* typus A Brion and Kayser 1902, 613.)

pa.ra.ty'phi. Gr. prep. *para* alongside of; Gr. n. *typhus* a stupor; M.L. gen. n. *paratyphi-A* of type A typhoid-like infection.

Antigenic formula: 1,2,12:a:–. As with other strains of O antigen groups A, B and D, the presence of factor 1 is connected with lysogenization.

Aerogenic. Ferments arabinose but no xylose.

The majority of strains do not produce H_2S, and in this respect "*S. paratyphi-A*" is unlike most other salmonellae.

Lysine decarboxylase is weak or negative.

Pathogenic only for man.

e. "*Salmonella schottmuelleri*" (Winslow et al., 1919) Bergey et al. 1923, 213. (*Bacterium paratyphi* typus B Brion and Kayser 1902, 613; *Bacillus schottmuelleri* Winslow, Kligler and Rothberg 1919, 479.)

schott.muel'ler.i. M.L. gen. n. *schottmuelleri* of Schottmüller; named after Prof. R. Schottmüller, who isolated the organism in 1899.

Antigenic formula; 1,4,[5],12:b:1,2.

Produces a slime layer when grown on a medium containing 0.5% glucose and 0.2 M sodium phosphate, pH 7 (Anderson, 1961).

Negative for *d*-tartrate.

Causes enteric fever in man and very rarely infects animals.

A variant known as *S. java* is positive for *d*-tartrate, fails to produce a slime layer, and usually causes enteritis in man and not uncommonly in animals as well (Kauffmann, 1941).

Some strains are intermediate between these two extremes.

f. *Salmonella typhimurium* (Loeffler 1892) Castellani and Chalmers 1919, 939.[AL] (*Bacillus typhimurium* Loeffler 1892, 134.)

ty.phi.mu'ri.um. Gr. n. *typhus* a stupor; L. n. *mus* mouse; l. gen. pl. n. *murium* of mice; M.L. gen. pl. n. *typhimurium* typhoid of mice.

Antigenic formula: 1,4,[5],12:i:1,2. The presence of factor 1 follows lysogenization by a converting phage named *iota* or PLT_{22}.

Ubiquitous and frequently the cause of infections in man and animals; also the most frequent agent of *Salmonella* gastroenteritis in man.

The well-known chromosome map of *Salmonella* is that of *S. typhimurium* strain LT_2 (for a review see Sanderson and Hartman, 1978).

Type strain: ATCC 13311.

g. *Salmonella enteritidis* (Gaertner 1888) Castellani and Chalmers 1919, 939.[AL] (*Bacillus enteritidis* Gaertner 1888, 573.)

en.te.ri'ti.dis. Gr. n. *enteron* gut, intestine; M.L. n. *enteritis* enteritis, inflammation of the intestine; M.L. gen. n. *enteritidis* of enteritis.

Antigenic formula: 1,9,12:g,m:–.

Frequently occurs in man and animals.

Type strain: ATCC 13076.

h. "*Salmonella gallinarum*" (Klein 1889) Bergey et al., 1925, 236. (*Bacillus gallinarum* Klein 1889, 689; *Bacterium pullorum* Rettger 1909, 123; *Salmonella gallinarum-pullorum* Taylor et al. 1952, 140.)

gal.li.na'rum. L. n. *gallina* hen; L. gen. pl. n. *gallinarum* of hens.

Antigenic formula: 1,9,12:–:–.

Always nonmotile. Maybe subdivided into biovars on the basis of fermentation characteristics, production of gas and production of H_2S.

Does not grow on a minimal defined medium.

Isolated chiefly from chickens and other birds. Causative agent of fowl typhoid.

"Subgenus" II

4. "*Salmonella salamae*" Le Minor, Rohde and Taylor 1970, 209. (*Salmonella dar-es-salaam* Salmonella Subcommittee 1934, 346.)

sa.la'mae. M.L. gen. n. *salamae* of (Dar-es) salaam.

Antigenic formula: 1,9,12:1,w:e,n,x.

Mucate and malonate positive; gelatin liquefaction slow.

Isolated in 1922 from the urine of a patient in Dar-es-Salaam (Tanzania) and the antigenic structure was determined by White (1926). Biochemical characteristics differ from previously identified salmonellae (Table 5.10) and the organism became the type of species of "subgenus" II.

Type strain: NCTC 5773 (ATCC 6959).

"Subgenus" III

j. *Salmonella arizonae* (Borman 1957) Kauffmann in van Oye, 1964.[AL] (*Paracolobactrum arizonae* Borman 1957, 347.)

(Text continues on p. 458)

Table 5.12.
Alphabetical list of names of **Salmonella** *serovars classified by "subgenus" and indicating the O group*

Serovar	O Group
"Subgenus" I	
S. aarhus	K
S. aba	C_2
S. abadina	M
S. abaetetuba	F
S. aberdeen	F
S. abidjan	Q
S. ablogame	I
S. abobo	I
S. abony	B
S. abortusbovis	B
"*S. abortuscanis*" 4,5,12:b:z_6 (phase R)	B
S. abortusequi	B
S. abortusovis	B
S. accra	E_4
S. adabraka	E_1
S. adamstown	M
S. adamstua	F
S. adana	U
S. adelaide	O
S. adeoyo	I
S. aderike	M
S. adime	C_1
S. adjame	G_2
S. aesch	C_2
S. aequatoria	C_1
S. aflao	H
S. africana	B
S. afula	C_1
S. agama	B
S. agbeni	G_2
S. agege	E_1
S. ago	N
S. agodi	O
S. agona	B
S. agoueve	G_1
S. ahanou	J
S. ahepe	U
S. ahmadi	E_4
S. ahuza	U
S. ajiobo	G_2
S. akanji	C_2
S. akuafo	I
S. alabama	D_1
S. alachua	O
S. alagbon	C_3
S. alamo	C_1
S. albany	C_3
S. albert	B
S. albuquerque	H
S. alexanderplatz	X
S. alexanderpolder	C_3
S. alfort	E_1
S. alger	P
S. allandale	R
S. allerton	E_1
S. alminko	C_3
S. altendorf	B
S. altona	C_3
S. amager	E_1
S. amba	F
S. amersfoort	C_1
S. amherstiana	C_3
S. amina	I
S. aminatu	E_1
S. amounderness	E_1
S. amoutive	M
S. amsterdam	E_1
S. amunigun	I
S. anatum	E_1
S. anderlecht	E_1
S. anecho	O
S. anfo	Q
S. angers	C_3
S. angoda	N
S. angouleme	I
S. anie	X
S. ank	M
S. anna	G_2
S. annedal	I
S. antarctica	D_1
S. antonio	57
S. antsalova	51
S. antwerpen	T
S. apapa	W
S. apeyeme	C_3
S. aqua	N
S. aragua	N
S. ardwick	C_4
S. arechavaleta	B
S. arkansas	E_3
S. aschersleben	N
S. ashanti	M
S. assen	L
S. assinie	E_1
S. atakpame	C_3
S. atento	F
"*S. atherton*" = *S. waycross*	S
S. athinai	C_1
S. atlanta (combined with *S. mississippi*)	G_2
S. augustenborg	C_1
S. austin	C_1
S. avignon	I
S. avonmouth	E_4
S. ayinde	B
S. ayton	B
S. azteca	B
S. babelsberg	M
S. babili	M
S. baguida	L
S. baguirmi	N
S. bahati	G_1
S. bahrenfeld	H
S. baiboukoum	C_1
S. baildon	D_2
S. bakau	M
S. balcones	W
S. ball	B
S. bama	J
S. bambesa (combined with *S. miami*)	D_1
S. bamboye	D_2
S. bambylor	D_2
S. banalia	C_2
S. banana	B
S. banco	M
S. bandia	O
S. bangkok	P
S. banjul	H
"*S. bantam*" = *S. meleagridis*	E_1
S. bardo	C_3
S. bareilly	C_1
S. bargny	C_3
S. barmbek	I
S. barranquilla	I
S. basingstoke	D_2
S. bassa	C_2
S. bassadji	M
"*S. batavia*" = *S. lexington*	E_1
S. battle	I
S. bazenheid	C_3
S. be	C_3
S. beaudesert	H

Table 5.12.—*continued*

Serovar	O Group
S. bedford	E_4
S. belem	C_2
S. belfast	C_2
S. benfica	E_1
S. benguella	R
S. benin	D_2
S. bere	X
S. bergedorf	D_2
S. bergen	X
S. berkeley	U
S. berlin	J
S. berta	D_1
S. bessi	E_1
S. biafra	E_1
S. bietri	N
S. bignona	J
S. bijlmer	R
S. bilu	E_4
S. bingerville	X
S. binningen	W
S. binza	E_2
S. birkenhead	C_1
S. birmingham	E_1
S. bispebjerg	B
S. blegdam	D_1
S. blijdorp	H
S. blitta	X
S. blockley	C_2
S. blukwa	K
S. bobo	V
S. bochum	B
S. bodjonegoro	N
S. boecker	H
S. bokanjac	M
S. bolombo	E_1
S. bolton	E_1
S. bonames	J
S. bonariensis	C_2
S. bonn	C_1
S. bootle	X
S. borbeck	G_1
S. bornum	C_4
S. borreze	54
S. bournemouth	D_1
S. bousso	H
S. bovismorbificans	C_2
S. bracknell	G_2
S. bradford	B
S. braenderup	C_1
S. brancaster	B
S. brandenburg	B
S. brazil	I
S. brazos	K
S. brazzaville	C_1
S. bredeney	B
S. brefet	V
S. breukelen	C_2
S. brevik	I
S. brezany	B
S. brijbhumi	F
S. brikama	C_3
S. brisbane	M
S. bristol	G_1
S. brive	T
S. bron	G_1
S. bronx	C_2
S. broughton	E_4
"S. broxbourne" = S. wien	B
S. bruck	C_1
S. brunei	C_3
S. budapest	B
"S. buenosaires" = S. bonariensis	C_2
S. bukavu	R
S. bukuru	C_2
S. bulgaria	C_2
S. bullbay	F
S. burgas	I
S. bury	B
S. businga	C_1
S. butantan	E_1
S. butare	52
S. buzu	H
S. caen	I
S. cairina	E_1
S. cairns	W
S. cairo (combined with *S. stanley*)	B
S. calabar	E_4
S. california	B
S. camberene	O
S. camberwell	D_1
S. cambridge	E_2
S. campinense	D_1
S. canada	B
S. cannonhill	E_4
S. cannstatt	E_4
S. canoga	E_3
S. canton	54
S. caracas	H
"S. cardiff" 6,7:k:1,10 (phase R)	C_1
S. carmel	J
S. carnac	K
S. carno	E_4
S. carrau	H
S. carswell	V
S. casablanca	W
S. casamance	R
S. catanzaro	H
S. cayar	C_1
S. cerro	K
S. ceyco	D_2
S. chagoua	G_2
S. chailey	C_2
S. champaign	Q
S. chandans	F
S. charity	H
S. charlottenburg	C_2
S. chester	B
S. chicago	M
S. chichiri	H
S. chincol	C_2
S. chingola	F
S. chiredzi	F
S. chittagong	E_4
S. choleraesuis	C_1
S. chomedey	C_3
S. christiansborg	V
S. clackamas	B
S. claibornei	D_1
S. clerkenwell	E_1
S. cleveland	C_2
S. clichy	E_2
S. cochin	D_2
S. cocody	C_3
S. coeln	B
S. coleypark	C_1
S. colindale	C_1
S. colobane	F
S. colombo	P
S. colorado	C_1
S. concord	C_1
S. congo	G_2
S. coogee	T
"S. cook" 39:z_{46}:1,5 (phase R)	Q
S. coquilhatville	E_1

Table 5.12.—*continued*

Serovar	O Group
S. corvallis	C_3
S. cotham	M
S. cotia	K
S. cremieu	C_2
S. croft	M
S. crossness	67
S. cubana	G_2
S. cuckmere	E_1
S. cullingworth	M
S. curacao	C_2
S. cyprus	C_2
S. dabou	C_3
S. dadzie	51
S. dahlem	Y
S. dahomey	X
S. dakar	M
S. dakota	I
S. dalat (combined with *S. ball*)	B
S. dallgow	E_4
S. dan	51
S. dapango	X
S. daytona	C_1
S. decatur (combined with *S. choleraesuis*)	C_1
S. dembe	O
S. demerara	G_2
S. denver	C_1
S. derby	B
S. derkle	52
S. dessau	E_4
S. deversoir	W
S. dieuppeul	M
S. diguel	G_1
S. diogoye	C_3
S. diourbel	L
S. djakarta	Y
S. djama	T
S. djelfa	C_3
S. djermaia	M
S. djibouti	J
S. djugu	C_1
S. doba	D_2
S. doncaster	C_2
S. donna	N
S. doorn	M
S. dougi	Z
S. doulassame	N
S. dresden	M
S. driffield	R
S. drogana	B
S. drypool	E_2
S. dublin	D_1
S. duesseldorf	C_2
S. dugbe	W
S. duisburg	B
S. dumfries	E_1
S. durban	D_1
S. durham	G_2
S. duval	R
S. ealing	O
S. eastbourne	D_1
S. eberswalde	D_1
S. eboko	C_2
S. ebrie	O
S. echa	P
S. edinburg	C_1
S. edmonton	C_2
S. egusi	S
S. egusitoo	T
S. eimsbuettel	C_4
S. eingedi	C_1
S. eko	B
S. ekotedo	D_2
S. ekpoui	X
S. elisabethville	E_1
S. elokate	D_1
S. elomrane	D_1
S. emek	C_3
S. emmastad	P
S. encino	H
S. enschede	O
S. entebbe	B
S. enteritidis	D_1
S. enugu	I
S. epicrates	E_1
S. epinay	F
S. eppendorf	B
S. escanaba	C_1
S. eschersheim	E_2
S. eschweiler	C_1
S. essen	B
S. etterbeek	F
S. ezra	M
S. fajara	M
S. faji	T
S. falkensee	E_1
S. fallowfield	E_1
S. fann	F
S. fanti	G_2
S. farakan	M
S. farcha	U
S. fareham	E_4
S. farmsen	G_2
S. fass	Z
S. fayed	C_2
S. ferlac	H
S. ferruch	C_3
S. findorff	F
S. finkenwerder	H
S. fischerhuette	I
S. fischerkietz	H
S. fischerstrasse	V
S. fitzroy	Y
S. florian	E_1
S. florida	H
S. flottbek	52
S. fluntern	K
S. fomeco	W
S. fortlamy	I
S. fortune	B
S. frankfurt	I
S. freetown	P
S. freiburg	E_1
S. fresno	D_2
S. friedenau	G_1
S. friendrichsfelde	M
S. frintrop	D_1
S. fufu	E_1
S. fulica	B
S. fyris	B
S. gabon	C_1
S. gafsa	I
S. galiema	C_1
S. galil	E_1
S. gallen	F
S. gallinarum	D_1
S. gamaba	V
S. gambaga	L
S. gambia	O
S. gaminara	I
S. garba	H
S. garoli	C_1
S. gassi	O
S. gateshead	D_2
S. gatineau	E_4

Table 5.12.—*continued*

Serovar	O Group
S. gatow	C_1
S. gatuni	C_2
S. gbadago	E_1
S. gdansk	C_1
S. gege	N
S. gelsenkirchen	C_4
S. georgia	C_1
S. gera	T
S. geraldton	D_2
S. ghana	L
S. giessen	N
S. give	E_1
S. giza	C_3
S. glasgow	I
S. glidji	F
S. glostrup	C_2
S. gloucester	B
S. gnesta	E_4
S. godesberg	N
S. goelzau	E_1
S. goerlitz	E_2
S. goeteborg	D_1
S. goettingen	D_1
S. gokul	51
S. goldcoast	C_2
S. goma	C_1
S. gombe	C_1
S. good	L
S. gori	J
S. goulfey	R
S. goverdhan	D
S. grampian	C_1
S. granlo	J
S. graz	U
S. greiz	R
S. groenekan	K
S. grumpensis	G_2
S. guarapiranga	N
S. guerin	D_2
S. guildford	M
S. guinea	V
S. gustavia	F
S. gwale	T
S. gwoza	E_4
S. haardt	C_3
S. hadar	C_2
S. haelsingborg	C_1
S. haferbreite	T
S. haga	O
S. haifa	B
S. halle	M
S. hallfold	B
S. halmstad	E_2
"*S. hamilton*" 3,*15*:e,h:1,2:z_{27} (phase R) (combined with *S. goerlitz*)	E_2
S. handen	G_2
S. hann	R
S. hannover	I
S. haouaria	G_1
S. harburg	H
S. harrisonburg	E_3
S. hartford	C_1
S. harvestehude	T
S. hatfield	M
S. hato	B
S. havana	G_2
S. heerlen	F
S. heidelberg	B
S. hermannswerder	M
S. heron	I
S. herston	C_2
S. herzliya	F
S. hessarek	B
S. heves	H
S. hidalgo	C_2
S. hiduddify	C_2
S. hillegersberg	D_2
S. hillingdon	D_2
S. hillsborough	C_1
S. hilversum	N
S. hindmarsh	C_3
S. hisingen	Y
S. hissar	C_4
S. hithergreen	I
S. hofit	Q
S. hoghton	E_1
S. holcomb	C_2
S. homosassa	H
S. honelis	M
S. horsham	H
S. huddinge	E_1
S. hull	I
S. huvudsta	E_1
S. hvittingfoss	I
S. hydra	L
S. ibadan	G_1
S. idikan	G_2
S. ikayi	E_1
S. ikeja	M
S. ilala	M
S. illinois	E_3
S. ilugun	E_4
S. inchpark	C_2
S. india	D_2
S. indiana	B
S. infantis	C_1
S. inganda	C_1
S. inglis	D_2
S. inpraw	S
S. inverness	P
S. ipeko	D_1
S. ipswich	S
S. irenea	J
S. irigny	U
S. irumu	C_1
S. isangi	C_1
S. isaszeg	Y
S. israel	D_1
S. istanbul	C_3
S. isuge	G_2
"*S. italiana*" 9,12:1,v:1,11 (phase R)	D_1
S. itami	D_1
S. ituri	B
S. itutaba	D_2
"*S. iwo-jima*" = *S. kentucky*	C_3
S. jaffna	D_1
S. jaja (combined with *S. stanleyville*)	B
S. jalisco	F
S. jamaica	D_1
S. jangwani	J
S. java (combined with *S. paratyphi B*)	B
S. javiana	D_1
S. jedburgh	E_1
S. jericho	B
S. jerusalem	C_4
S. joal	E_1
S. jodhpur	W
S. joenkoeping (combined with *S. kingston*)	B
S. johannesburg	R
S. jos	B
S. juba	E_4
S. jubilee	J
S. jukestown	G_2
S. kaapstad	B

Table 5.12.—*continued*

Serovar	O Group
S. kabete	51
S. kaduna	C_4
S. kahla	T
S. kaitaan	H
S. kalamu	B
S. kalina	E_1
S. kalumburu	C_2
S. kambole	C_1
S. kamoru	B
S. kampala	T
"S. kanda" = S. meleagridis	E_1
S. kande	E_4
S. kandla	J
S. kaneshie	T
S. kano	B
S. kaolack	X
S. kapemba	D_1
S. kaposvar (combined with *S. reading*)	B
S. karachi	W
S. karamoja	R
S. kasenyi	P
S. kassberg	H
S. kedougou	G_2
S. kentucky	C_3
S. kenya	C_1
S. kermel	V
S. keve	L
S. khartoum	E_3
S. kiambu	B
S. kibi	I
S. kibusi	M
S. kidderminster	P
S. kiel	A
S. kikoma	I
S. kimberley	P
S. kimpese	D_2
S. kimuenza	B
S. kingabwa	U
S. kingston	B
S. kinondoni	J
S. kinshasa	E_2
S. kintambo	G_2
S. kirkee	J
S. kisangani	B
S. kisarawe	F
S. kisii	C_1
S. kitenge	M
S. kivu	C_1
S. klouto	P
S. kodjovi	X
S. koenigstuhl	B
S. koketime	V
S. kokoli	N
S. kokomlemle	Q
S. konstanz	C_3
S. korbol	C_3
S. korlebu	E_4
S. korovi	P
S. kortrijk	C_1
S. kottbus	C_2
S. kotte	C_1
S. kouka	E_4
S. koumra	C_1
S. kpeme	M
S. kralingen	C_3
S. krefeld	E_4
S. kristianstad	E_1
S. kua	V
S. kubacha	B
S. kuessel	M
S. kumasi	N
S. kunduchi	B
S. kuru	C_2
S. labadi	C_3
S. lagos	B
S. lamin	E_1
S. landala	S
S. landau	N
S. landwasser	E_1
S. langenhorn	K
S. langensalza	E_1
S. langford	M
S. lanka	E_2
S. lansing	P
S. larochelle	C_1
S. lattenkamp	W
S. lawndale	D_1
S. lawra	V
S. leatherhead	S
S. lechler	51
S. leeuwarden	F
S. legon	B
S. leiden	G_1
S. leipzig	S
S. leith	C_2
S. lekke	E_1
S. lene	F
S. leoben	M
S. leopoldville	C_1
S. lerum	E_4
S. lexington	E_1
S. lezennes	C_2
S. ligeo	N
S. ligna	O
S. lika	C_1
S. lille	C_1
S. limete	B
S. lindenburg	C_2
S. lindern	H
S. lindi	P
S. linguere	D_2
S. lingwala	I
S. linton	G_2
S. lisboa	I
S. lishabi	D_2
S. litchfield	C_2
S. liverpool	E_4
S. livingstone	C_1
S. livulu	N
S. ljubljana	B
S. llandoff	E_4
S. loanda	C_2
S. lockleaze	C_4
S. lode	J
S. lodz	S
S. loenga	T
S. logone	Q
S. lokstedt	E_4
S. lomalinda	D_1
S. lome	D_1
S. lomita	C_1
S. lomnava	I
S. london	E_1
S. losangeles	I
S. louga	N
S. louisiana	D_2
S. lovelace	G_1
S. lubumbashi	S
S. luciana	F
S. luckenwalde	M
S. luke	X
S. lyon	X
S. maastricht	F
S. macallen	E_1

Table 5.12.—*continued*

Serovar	O Group
S. machaga	E_4
S. madelia	H
S. madiago	E_4
S. madigan	V
S. madison	L
S. madjorio	E_1
S. magumeri	H
S. magwa	L
S. maiduguri	E_4
S. makiso	C_1
S. malakal	I
S. malaysia	M
S. malika	V
S. malmoe	C_2
S. malstatt	I
S. mampeza	H
S. mampong	G_1
S. manchester	C_2
S. mandera	I
S. mango	P
S. manhattan	C_2
S. manila	E_2
S. mapo	C_2
S. mara	Q
S. maracaibo	F
S. maregrosso	66
S. maricopa	T
S. marienthal	E_1
S. maron	E_1
S. marseille	F
S. marshall	G_1
S. maryland	57
S. marylebone	D_2
S. masembe	E_1
S. massakory	O
S. massenya	B
S. matadi	J
S. mathura	D_2
S. matopeni	N
S. mayday	D_2
S. mbandaka	C_1
S. mbao	U
S. meekatharra	W
S. meleagridis	E_1
S. memphis	K
S. menden	C_1
S. mendoza	D_1
S. menhaden	E_3
S. menston	C_1
S. mesbit	X
S. meskin	51
S. messina	N
S. mexicana (combined with *S. muenchen*)	C_2
S. mgulani	P
S. miami	D_1
S. michigan	J
S. middlesbrough	T
S. midway	H
S. mikawasima	C_1
S. millesi	R
S. milwaukee	U
S. mim	G_1
S. minna	H
S. minneapolis	E_3
S. minnesota	L
S. mishmarhaemek	G_2
S. mission (combined with *S. isangi*)	C_1
S. mississippi	G_2
S. miyazaki	D_1
S. mkamba	C_1
S. mocamedes	M
S. moero	M
S. mokola	E_1
S. molade	C_3
S. molesey	52
S. mono	B
S. mons	B
S. monschaui	O
S. montevideo	C_1
S. montreal	U
S. morehead	N
S. morningside	N
S. mornington	H
S. morocco	N
S. morotai	J
S. moroto	M
S. moscow	D_1
S. mowaline	X
S. mountpleasant	X
S. moussoro	H
S. mowanjum	C_2
S. mpouto	I
S. muenchen	C_2
S. muenster	E_1
S. muguga	V
S. mundonobo	M
S. mura	B
S. naestved	D_1
S. nagoya	C_2
S. nakuru	B
S. nancy	E_2
S. nanergou	C_2
S. nanga	G_2
S. napoli	D_1
S. narashino	C_2
S. nashua	M
S. naware	I
S. nchanga	E_1
S. ndjamena	H
S. ndolo	D_1
S. neftenbach	B
S. nessa	H
S. nessziona	C_1
S. neudorf	N
S. neukoelln	C_1
S. neumuenster	B
S. newbrunswick	E_2
S. newhaw	E_2
S. newington	E_2
S. newlands	E_1
S. newmexico	D_1
S. newport	C_2
S. newrochelle	E_1
S. newyork	G_1
S. ngili	C_1
S. ngor	E_4
S. niakhar	V
S. niamey	J
S. niarembe	V
S. nienstedten	C_4
S. nieukerk	C_4
S. nigeria	C_1
S. nijmegen	N
S. nikolaifleet	I
S. niloese	E_4
S. nima	M
S. nimes	G_1
S. nissii combined with *S. nienstedten*)	C_4
S. nitra	A
S. njala	P
S. nordufer	C_2
S. norton	C_1
S. norwich	C_1
S. nottingham	I

Table 5.12.—*continued*

Serovar	O Group
S. nowawes	R
S. nuatja	I
S. nyanza	F
S. nyborg	E_1
S. nyeko	I
S. oakland	C_1
S. obogu	C_1
S. ochsenwerder	54
S. odozi	N
S. oerlikon	Q
S. oevelgoenne	M
S. offa	S
S. ogbete	U
S. ohio	C_1
S. ohlstedt	E_1
S. okatie	G_2
S. okefoko	E_1
S. okerara	E_1
S. oldenburg	I
S. olten	D_2
S. omderman	C_4
S. omifisan	R
S. ona	M
S. onarimon	D_1
S. onderstepoort	H
S. onireke	E_1
S. ontario	D_2
S. oranienburg	C_1
S. ord	52
S. ordonez	G_2
S. oregon (combined with *S. muenchen*)	C_2
S. orientalis	I
S. orion	E_1
S. oritamerin	C_1
S. orlando	K
S. os	D_1
S. oskarshamn	M
S. oslo	C_1
S. osnabrueck	F
S. othmarschen	C_1
S. ottawa	D_1
S. ouakam	D_2
S. oudwijk	G_1
S. overchurch	R
S. overschie	51
S. overvecht	N
S. oxford	E_1
S. oyonnax	C_1
S. pakistan	C_3
S. palime	C_1
S. panama	D_1
S. pankow	E_2
S. papuana	C_1
S. paratyphi A	A
S. paratyphi B = *S. schottmuelleri*	B
S. paratyphi C = *S. hirschfeldii*	C_1
S. paris	C_3
S. parkroyal	E_4
S. pasing	B
S. patience	M
S. penarth	D_1
S. pensacola	D_1
S. perth	P
S. pharr	F
S. pikine (combined with *S. altona*)	C_3
S. pisa	I
S. plymouth	D_2
S. poano	H
S. poeseldorf	54
S. pomona	M
S. pontypridd	K
S. poona	G_1
S. portland	D_1
S. portsmouth	E_2
S. potosi	H
S. potsdam	C_1
S. potto	D_2
S. pramiso	E_1
S. praha	C_2
S. presov	C_2
S. preston	B
S. pretoria	F
S. pueris (combined with *S. newport*)	C_2
S. pullorum	D_1
S. putten	G_2
S. quebec	V
S. quentin	D_2
S. quinhon	X
S. quiniela	C_2
S. ramatgan	N
S. ramsey	M
S. raus	G_1
S. rawash	K
S. reading	B
S. rechóvot	C_3
S. redba	C_1
S. redhill	F
S. redlands	I
S. regent	E_1
S. reinickendorf	B
S. remete	F
S. remo	B
S. reubeuss	C_3
S. rhone	L
S. rhydyfelin	I
S. richmond	C_1
S. rideau	E_4
S. ridge	D_1
S. ried	G_1
S. riggil	C_1
S. riogrande	R
S. rissen	C_1
S. rittersbach	P
S. riverside	W
S. roan	P
S. rochdale	Z
S. rogy	M
S. romanby	G_2
S. roodepoort	G_1
S. rosenthal	E_2
S. rossleben	54
S. rostock	D_1
S. rottnest	G_1
S. rovaniemi	I
S. rubislaw	F
S. ruiru	L
S. ruki (combined with *S. ball*)	B
S. rumford	C_1
S. runby	H
"S. rutgers" 3,10:l,z_{40}:1,7 (phase R)	E_1
S. rruzizi	E_1
S. saarbruecken	D_1
S. saboya	I
S. sada	N
S. sainte-marie	52
S. saint-paul	B
S. saka	X
"S. sakai" = *S. potsdam*	C_1
S. salford	I
S. salinatis	B
S. saloniki	I
S. sambre	E_4
S. sandiego	B
S. sandow	C_2

Table 5.12.—*continued*

Serovar	O Group
S. sanga	C_3
S. sangalkam	D_2
S. sangera	I
S. sanjuan	C_1
S. sanktgeorg	M
S. sanktmarx	E_4
S. santhiaba	R
S. santiago	C_3
S. sao	E_4
S. saphra	I
S. sara	H
S. sarajane	B
S. saugus	R
S. schalkwijk	H
S. schleissheim	B
S. schoeneberg	E_4
"S. schottmuelleri" = S. paratyphi B	B
S. schwarzengrund	B
S. schwerin	C_2
S. seattle	M
S. sedgwick	V
S. seegefeld	E_1
S. sekondi	E_1
S. selandia	E_2
S. selby	M
S. sendai	D_1
S. senegal	F
S. senftenberg	E_4
S. seremban	D_1
S. shamba	I
S. shangai	I
S. shangani	E_1
S. shannon	E_1
S. sharon	F
S. sherbrooke	I
S. sheffield	P
S. shikmonah	R
S. shipley	C_3
S. shomolu	M
S. shoreditch	D_2
S. shubra	B
S. simi	E_1
"S. simsbury" 1,3,19:z_{27}:- (phase R)	E_4
S. sinchew	E_1
S. singapore	C_1
S. sinstorf	E_1
S. sinthia	K
S. sipane	T
S. skansen	C_2
S. sladun (combined with *S. abony*)	B
S. sljeme	X
S. sloterdijk	B
S. soahanina	H
S. soerenga	N
S. sokode	D_2
S. solna	M
S. solt	F
S. somone	C_1
S. southampton	B
S. southbank	E_1
S. souza	E_1
S. spartel	L
S. stanley	B
S. stanleyville	B
S. staoueli	X
S. steinplatz	N
S. steinwerder	54
S. stellingen	X
S. stendal	F
S. sternschanze	N
S. sterrenbos	C_2
S. stockholm	E_1
S. stormont	E_1
S. stourbridge	C_2
S. straengnaes	F
S. strasbourg	D_2
S. stratford	E_4
S. stuivenberg	E_4
S. suberu	E_1
S. suelldorf	W
"S. suez" = S. shubra	B
"S. suipestifer" = S. choleraesuis	C_1
S. sundsvall	H
S. sunnycove	C_3
S. surat	H
S. sya	X
S. szentes	I
S. tabligbo	X
S. tado	C_3
S. tafo	B
"S. taihoku" = S. meleagridis	E_1
S. takoradi	C_2
S. taksony	E_4
S. tallahassee	C_2
S. tamale	C_3
S. tambacounda	E_4
S. tamberma	X
S. tamilnadu	C_1
S. tananarive	C_2
S. tanger	G_1
S. tanzania	G_1
S. tarshyne	D_1
S. taset	T
S. taunton	M
S. tchad	O
S. tchamba	J
S. techimani	M
S. teddington	B
S. tees	I
S. tejas	B
S. teko	H
S. telaviv	M
S. telelkebir	G_2
S. telhashomer	F
S. teltow	M
S. tennessee	C_1
S. tennyson	B
S. teshie	X
S. texas	B
S. thaygen	B
S. thetford	U
S. thiaroye	P
S. thielallee	C_4
S. thomasville	E_3
S. thompson	C_1
S. tiergarten	V
S. tilburg	E_4
S. tilene	R
S. tim (combined with *S. newington*)	E_2
S. tinda	B
S. tione	51
S. togba	I
S. togo	B
S. tokoin	B
S. tomegbe	T
S. tomelilla	E_4
S. tonev	54
S. toowong	F
S. toricada	T
S. tornow	W
S. toronto	D_2
S. toucra	Y
S. toulon	K
S. tounouma	C_3

Table 5.12.—*continued*

Serovar	O Group
S. tournai	E_2
S. trachau	B
S. travis	B
S. treforest	51
S. trimdon	D_2
S. trotha	R
S. truro	E_1
S. tschangu	G_2
S. tsevie	B
S. tshiongwe	C_2
S. tucson	H
S. tudu	B
S. tuebingen	E_2
S. tunis	G_2
S. typhi	D_1
S. typhimurium	B
S. typhisuis	C_1
S. tyresoe	B
S. uccle	54
S. uganda	E_1
S. ughelli	E_1
S. uhlenhorst	V
S. uithof	52
S. ullevi	G_2
S. umbilo	M
S. umhlali	C_1
S. umhlatazana	O
S. uno	C_2
S. uppsala	B
S. urbana	N
S. ursenbach	T
S. usumbura	K
S. utah	C_2
S. utrecht	52
S. uzaramo	H
S. vaertan	G_1
S. vancouver	I
S. vejle	E_1
S. vellore	B
S. veneziana	F
S. venusberg (combined with *S. nchanga*)	E_1
S. victoria	D_1
S. victoriaborg	J
S. vietnam	S
S. vilvoorde	E_4
S. vinohrady	M
S. virchow	C_1
S. virginia	C_3
S. visby	E_4
S. vitkin	M
S. vleuten	V
S. vogan	T
S. volksmarsdorf	M
S. volta	F
S. vom	B
S. wagenia	B
S. wandsworth	Q
S. wangata	D_1
S. waral	T
S. warengo	J
S. warnemuende	M
S. warnow	C_2
S. warragul	H
S. washington	G_1
S. waycross	S
S. wayne	N
S. wedding	M
S. welikade	I
S. weltevreden	E_1
S. wentworth	F
S. wernigerode	D_2
S. weslaco	T
S. westeinde	I
S. westerstede	E_4
S. westhampton	E_1
S. westminster	E_2
S. weston	I
S. westphalia	O
S. weybridge	E_1
S. wichita	G_2
S. widemarsh	O
S. wien	B
S. wil	C_1
S. wildwood	E_3
S. wilhelmsburg	B
S. willemstad	G_1
S. wimborne	E_1
S. windermere	Q
S. wingrove	C_2
S. winnipeg	54
S. winterthur	E_4
S. wippra	C_2
S. wisbech	I
S. wohlen	F
S. womba (combined with *S. altendorf*)	B
S. worb	D_2
S. worthington	G_2
S. wuerzburg (combined with *S. miami*)	D_1
S. wuiti	N
S. wuppertal	D_2
S. wyldegreen	G_2
S. yaba	E_1
S. yalding	E_4
S. yaounde	B
S. yarm	C_2
S. yarrabah	G_2
S. yeerongpilly	E_1
S. yehuda	F
S. yerba	54
S. yoff	P
S. yokoe	C_3
S. yolo	O
S. yovokome	C_3
S. yundum	E_1
S. zadar	D_2
S. zagreb (combined with *S. saintpaul*)	B
S. zaire	N
S. zanzibar	E_1
S. zega	D_1
S. zehlendorf	N
S. zerifin	C_2
S. zongo	E_1
S. zuilen	E_4
S. zwickau	I
Subgenus" II	
S. II *acres*	G_2
S. II *alexander*	E_1
S. II *alsterdorf*	R
S. II *angola*	D_1
S. II *artis*	56
S. II *askraal*	51
S. II *atra*	Z
S. II *bacongo*	C_1
S. II *baragwanath*	C_2
S. II *basel*	58
S. II *bechuana*	B
S. II *bellville*	I
S. II *beloha*	K
S. II *betioky*	59
S. II *bilthoven*	X
S. II *blankenese*	D_1
S. II *bleadon*	J
S. II *bloemfontein*	C_1
S. II *boksburg*	R

Table 5.12.—*continued*

Serovar	O Group
S. II *bornheim*	H
S. II *boulders*	G_2
S. II *bremen*	W
S. II *bulawayo*	R
S. II *bunnik*	U
S. II *caledon*	B
S. II *calvinia*	C_1
S. II *canastel*	D_1
S. II *cape*	C_1
S. II *carletonville*	P
S. II *ceres*	M
S. II *chersina*	X
S. II *chinovum*	T
S. II *chudleigh*	E_1
S. II *clifton*	G_1
S. II *clovelly*	V
S. II *constantia*	J
S. II *daressalaam*	D_1
S. II *degania*	R
S. II *detroit*	T
S. II *dubrovnik*	S
S. II *duivenhoks*	D_2
S. II *durbanville*	B
S. II *eilbek* (combined with *S.* III *arizonae* 61:i:z)	61
S. II *ejeda*	W
S. II *elsiesrivier*	I
S. II *emmerich*	H
S. II *epping*	G_2
S. II *erlangen*	Y
S. II *fandran*	R
S. II *faure*	Z
S. II *finchley*	E_1
S. II *foulpointe*	P
S. II *fremantle*	T
S. II *fuhlsbuettel*	E_1
S. II *germiston*	C_2
S. II *gilbert*	C_1
S. II *glencairn*	F
S. II *gojenberg*	G_2
S. II *goodwood*	G_1
S. II *grabouw*	F
S. II *greenside*	Z
S. II *grunty*	R
S. II *gwaai*	L
S. II *haarlem*	D_2
S. II *haddon*	I
S. II *hagenbeck*	Y
S. II *hamburg* (combined with *S.* II 1,9,12:g,m[s],t:[1,5]:[z_{42}])	D_1
S. II *hammonia*	Y
S. II *heilbron*	C_1
S. II *helsinki*	B
S. II *hennepin*	S
S. II *hillbrow*	J
S. II *hooggraven*	Z
S. II *hueningen*	D_1
S. II *huila*	F
S. II *humber*	53
S. II *islington*	E_1
S. II *jacksonville*	I
S. II *kaltenhausen*	M
S. II *katesgrove*	G_2
S. II *khami*	X
S. II *kilwa*	B
S. II *klapmuts*	W
S. II *kluetjenfelde*	B
S. II *kommetje*	U
S. II *kraaifontein* (combined with *S.* II *luanshya*)	G_2
S. II *krugersdorp*	Z
S. II *kuilsrivier*	D_1
S. II *lethe*	S
S. II *lichtenberg*	S
S. II *limbe*	G_1
S. II *lincoln*	F
S. II *lindrick*	D_1
S. II *llandudno*	M
S. II *lobatsi*	52
S. II *locarno*	57
S. II *louwbester*	I
S. II *luanshya*	G_2
S. II *lundby*	D_2
S. II *lurup*	S
S. II *luton*	60
S. II *maarssen*	D_2
S. II *makoma*	B
S. II *makumira*	B
S. II *manica* (combined with *S.* II 1,9,12:g,m,[s],t:[1, 5]:[z_{42}])	D_1
S. II *manombo*	57
S. II *matroosfontein*	E_1
S. II *merseyside*	I
S. II *midhurst*	53
S. II *mjimwema*	D_1
S. II *mobeni*	I
S. II *mondeor*	Q
S. II *montgomery*	F
S. II *mosselbay*	U
S. II *mpila*	E_1
S. II *muizenberg* (combined with S. II 1,9,12:g,m,[s],t:[1, 5]:[z_{42}])	D_1
S. II *nachshonim*	G_2
S. II *nairobi*	T
S. II *namib*	Z
S. II *neasden*	D_1
S. II *negev*	S
S. II *ngozi*	Y
S. II *noordhoek*	I
S. II *nordenham*	B
S. II *neurnberg*	T
S. II *odijk*	N
S. II *ottershaw*	R
S. II *oysterbeds*	C_1
S. II *parow*	E_2
S. II *perinet*	W
S. II *phoenix*	X
S. II *portbech*	T
S. II *quimbamba*	X
S. II *rand*	T
S. II *rhodesiense*	D_1
S. II *roggeveld*	51
S. II *rooikrantz*	H
S. II *rotterdam*	G_1
S. II *rowbarton*	I
S. II *sakaraha*	Y
S. II *sarepta*	I
S. II *seaforth*	Z
S. II *setubal*	60
S. II *shomron* (combined with *S.* III *arizonae* 18:z_4,z_{32}:-)	K
S. II *simonstown*	H
S. II *slangkop*	H
S. II *slatograd*	N
S. II *sofia*	B
S. II *soutpan*	F
S. II *springs*	R
S. II *srinagar*	F
S. II *stellenbosch*	D_1
S. II *stevenage*	G_2
S. II *stikland*	E_1
S. II *suarez*	R
S. II *suederelbe*	D_1
S. II *sullivan*	C_1
S. II *sunnydale*	R
S. II *sydney* (combined with *S.* III *arizonae* 48:i:z)	Y
S. II *tafelbaai*	E_1
S. II *tokai*	57
S. II *tosamanga*	C_1
S. II *tranoroa*	55

Table 5.12.—*continued*

Serovar	O Group
S. II *tulear*	C_2
S. II *tygerberg*	G_2
S. II *uphill*	T
S. II *utbremen*	O
S. II *veddel*	U
S. II *verity*	J
S. II *vredelust*	G_2
S. II *vrindaban*	W
S. II *wandsbek*	L
S. II *westpark*	E_1
S. II *wilhemstrasse* (combined with *S.* II *lobatsi*)	52
S. II *winchester*	E_1
S. II *windhoek*	W
S. II *woerden*	J
S. II *woodstock*	I
S. II *worcester*	G_2
S. II *wynberg*	D_1
S. II *zeist*	K
S. II *zuerich*	D_3
"Subgenus" IV	
S. IV *argentina*	C_1
S. (IV) *bern* (combined with *S.* IV 40:z_4, z_{32})	R
S. IV *bockenheim*	53
S. IV *bonaire*	Z
S. IV *chameleon*	I
S. IV *flint*	Z
S. IV *harmelen*	51
S. IV *houten*	U
S. IV *kralendyk*	C_1
S. IV *lohbruegge*	V
S. IV *marina*	Y
S. IV *mundsburg*	F
S. IV *ochsenzoll*	I
S. IV *parera*	F
S. IV *roterberg*	C_1
S. IV *sachsenwald*	R
S. IV *seminole*	R
S. IV *soesterberg*	L
S. IV *tuindorp*	U
S. IV *volksdorf*	U
S. IV *wassenaar*	Z
"Subgenus" V	
S. V *balboa*	Y
S. V *bongor*	Y
S. V *brookfield*	66
S. V *camdeni*	V
S. V *malawi*	66
S. V *maregrosso*	66

a.ri.zo'nae. M.L. gen. n. *arizonae* of Arizona, a state in the United States.

Antigenic formula: 51:z_4,z_{23}:–. (The corresponding "*Arizona*" formula is 1,2,:1,2,5:–.)

The original strains isolated from reptiles were designated dar-es-Salaam type var. from Arizona (Caldwell and Ryerson, 1939). The antigenic formula was determined by Kauffman (1941) as 33:z_4,z_{23},$_{36}$:–, and he gave it the name *Salmonella* sp. (serotype) *arizona*. After Edwards et al. (1947) established *Arizona* as an independent group, the O antigen 33 was deleted from the Kauffmann-White scheme. O antigen 51 is identical with the old O antigen 33 and with the *Arizona* antigen designated 1,2 by Edwards et al. The H antigens z_4, z_{23}, z_{36} (simplified to z_4, z_{23}) correspond to H antigens 1, 2, 5 of Edwards et al.

Type strain: ATCC 13314 (NCTC 9297).

"Subgenus" IV

k. "*Salmonella houtenae*" Le Minor, Rohde and Taylor 1970, 209. (*Salmonella houten* Kauffmann 1962, 353.)

hou'te.nae. M.L. gen. n. *houtenae* of Houten, a town in Holland.

Antigenic formula: 43:z_4,z_{23}:–.

The type species of *Salmonella* "subgenus" IV. It is the oldest known member of the "subgenus" (see discussion by Kauffmann, 1966, p. 244 on *S. delplata*, a mixed culture from which the serovar *S. houtenae* was obtained).

Type strain: NCTC 10401.

"Subgenus" V

l. "*Salmonella bongor*" Le Minor, Chamoiseau, Chairé-Marsaines and Egrou 1969, 775.

bon'gor. M.L. n. *bongor* Bongor, a town in Chad.

Antigenic formula: 48:z_{35}:–.

It is the oldest known member of the "subgenus." Isolated from a lizard in Chad.

Genus IV. **Citrobacter** *Werkman and Gillen 1932, 173*[AL]

RIICHI SAKAZAKI

Cit.ro.bac'ter. L. n. *citrus* lemon; M.L. n. *bacter* a small rod; M.L. masc. n. *Citrobacter* a citrate-utilizing rod.

Straight rods, ~1.0 μm in diameter and 2.0–6.0 μm in length. Occur singly and in pairs. Conform to the general definition of the family *Enterobacteriaceae*. Usually not encapsulated. Gram-negative. **Usually motile by peritrichous flagella.** Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Grow readily on ordinary media. Colonies on nutrient agar are generally 2–4 mm in diameter, smooth, low convex, moist, translucent or opaque and gray with a shiny surface and entire edge. Mucoid or rough forms may occur occasionally. Oxidase-negative. Catalase-positive. Chemoorganotrophic. **Citrate can be utilized as a sole carbon source.** Nitrate is reduced to nitrite. **Lysine is not decarboxylated.** Phenylalanine deaminase, gelatinase, lipase and deoxyribonuclease are not produced. Alginate and pectate are not decomposed. Glucose is fermented with the production of acid and gas. The methyl red test is positive; **the Voges-Proskauer test is negative.** Occur in the feces of man and other animals; probably normal intestinal inhabitants. Often isolated from clinical specimens as opportunistic pathogens. Also found in soil, water, sewage and food. The mol% G + C of the DNA is 50–52 (T_m).

Type species: *Citrobacter freundii* Werkman and Gillen 1932, 173.

Further Descriptive Information

Members of *Citrobacter* may or may not ferment lactose promptly but nearly always produce β-galactosidase. L-Arabinose, cellobiose, maltose, L-rhamnose, trehalose, D-xylose, D-mannitol, D-sorbitol, and glycerol are fermented rapidly by the majority of strains. Raffinose and *myo*-inositol are rarely attacked.

Ornithine is decarboxylated by almost all strains of *C. diversus* and *C. amalonaticus*, but less than 20% of strains of *C. freundii* produce this enzyme. Strains of *C. freundii* and *C. amalonaticus* in contrast to *C. diversus* can grow in media containing potassium cyanide.

Strains of *C. diversus* ferment D-adonitol, but nearly all strains of *C. freundii* and *C. amalonaticus* fail to ferment this substrate. Malonate is utilized as a sole carbon source by most strains of *C. diversus*, but can be used by less than 15% of the strains of *C. freundii* and not by *C. amalonaticus*.

The majority of strains of *C. freundii* produce abundant H_2S in the butt of Kligler iron agar and triple-sugar iron agar. Lactose is fermented by many strains of *C. freundii*, but the reactions are frequently delayed.

Indole is not produced by *C. freundii* with few exceptions, but all strains of *C. diversus* and *C. amalonaticus* give a positive indole test.

Nitrogen fixation under anaerobic condition has been reported in some strains of *C. freundii* isolated from the hindgut of Australian termites and from paper mill process water (Bergensen, 1980).

West and Edwards (1954) first established an antigenic schema of the Bethesda-Ballerup group of bacteria, which is now called *C. freundii*, based on their early studies (Edwards et al., 1948; Bruner et al., 1949; Moran and Bruner, 1949). The antigenic schema included 32 O groups and 87 H antigens. Sedlák and Slajsová (1966, 1967) and Sedlák (1974) expanded the antigenic schema by adding further O and H antigens, increasing the total number to 42 O and more than 90 H antigens. The antigens of many serovars of *C. freundii* relate to those of many *Salmonella* and *Escherichia* cultures (West and Edwards, 1954; Sakazaki and Namioka, 1957; Davis and Ewing, 1963; Sedlák and Slajsová, 1966). O antigenic relationships between *C. freundii* and *Hafnia alvei* were reported by Sakazaki (1971) and Sedlák and Slajsová (1966). The H antigens of *C. freundii* are monophasic. Some strains of O groups 5 and 29 of *C. freundii* may possess an antigen serologically identical with the Vi antigen of *Salmonella typhi* (Kauffmann and Møller, 1940; Monteverde, 1944). In contrast to *S. typhi*, however, quantitative variation of the Vi antigen in *C. freundii* cultures is reversible, and the presence of the Vi antigen is not related to the virulence of the cultures.

Serological studies of *C. diversus* were first reported by Gross et al. (1973) using four isolates from infantile meningitis. Later, Gross and Rowe (1974, 1975) and Gross et al. (1981) designated 17 O groups without any account of the H antigens. Popoff and Richard (1975), who studied the serology of *C. diversus* independently of Gross and Rowe, established an antigenic schema which contained 6 O groups and 7 H antigens. Sourek and Aldová (1976) also studied O antigens of *C. diversus* and independently proposed 9 O antigens.

Although no antigenic schema was proposed, van Oye et al. (1975) reported that the O antigens of 35 of 38 strains of *C. amalonaticus* were closely related to those of several serovars of *Shigella dysenteriae* and *Shigella boydii*. Sourek and Aldová (1976) presented an O grouping system in which 13 O groups were designated.

Members of *C. freundii* are usually susceptible to the aminoglycosides, chloramphenicol and colistin. Susceptibility of *C. freundii* to ampicillin, tetracycline and the cephalosporins differs among the strains. *C. diversus* and *C. amalonaticus* are susceptible to the aminoglycosides, cephalosporins, colistin, chloramphenicol and tetracycline. *C. diversus* and *C. amalonaticus* generally appear to be resistant to ampicillin and carbenicillin (Lund et al. 1974).

Members of the genus *Citrobacter* occur not only in feces of man and other animals with no disorder but also in water, sewage, soil and food. They are also found in clinical bacteriology not only in stools but also in urine, sputum and specimens from bacteremia, meningitis, otitis media, wounds, abscesses, the throat and autopsies; their role seems to be that of an opportunistic pathogen. Recently cases of neonatal meningitis caused by *C. diversus* have often been reported (Gross et al., 1973; Gwynn and George, 1973; Puentes et al., 1975; Tamborlane and Soto, 1975; Ribeiro et al., 1976). Although *C. freundii* was once considered to be an enteropathogen, it seems rather to be a normal inhabitant of the intestine (Sakazaki et al., 1960). Some investigators, however, have suggested a possible role of certain strains of *C. freundii* and *C. diversus* in causing diarrhea (Kleinmeier and Schafer, 1956; Sakazaki and Namioka, 1957; Sedlák, 1957; Nestorescu et al., 1964; Popovic et al., 1964; Guerrant et al., 1976; Wadström et al., 1976; Finn, 1978).

Enrichment and Isolation Procedures

The majority of *C. freundii* strains can grow in liquid enrichment media such as selenite broth and tetrathionate broth and on selective isolation media such as salmonella-shigella agar, deoxycholate-citrate agar, brilliant green agar and bismuth sulfite agar. Colonies which ferment lactose slowly can resemble *Salmonella* colonies in many instances.

Although *C. diversus* and *C. amalonaticus* strains are usually able to grow on the selective media indicated above, many strains are inhibited to some extent; therefore, less inhibitory media such as MacConkey agar and xylose-lysine-deoxycholate agar may be preferable.

Maintenance Procedures

Stock cultures of *Citrobacter* strains may be maintained at room temperature in a semisolid medium containing 1.0% Bacto-casitone (Difco), 0.3% yeast extract, 0.5% NaCl and 0.3% agar, pH 7.0. The cultures remain viable up to a year without subculturing if they are sealed with a rubber stopper or a cork which has been soaked in hot paraffin wax. Strains may also be preserved indefinitely by lyophilization.

Differentiation of the genus **Citrobacter** *from other genera*

Table 5.13 indicates the characteristics of *Citrobacter* that differentiate it from biochemically similar genera.

Taxonomic Comments

The genus *Citrobacter* was proposed by Werkman and Gillen (1932) for the citrate-utilizing "coli-aerogenes intermediates." Until recent years, however, the name did not gain acceptance and the organisms have been described under a variety of designations. *C. freundii* was described as "*Escherichia freundii*" by Yale (1939), and as "*Colobactrum freundii*" (for rapid lactose fermenters) and *Paracolobactrum intermedium* (for slow lactose fermenters) by Borman et al. (1944). The role of citrobacters as possible pathogens was first noticed by Kauffmann and Møller (1940), who described an organism called "*Salmonella ballerup*" which is presently classified in *C. freundii*. Monteverde (1944) reported an organism similar to *S. ballerup* under the name "*Salmonella hormaechei*." Later, this biogroup of organisms was removed from the genus *Salmonella* and was called the Ballerup group (Harhoff, 1949; Bruner et al., 1949). Independently of the Ballerup group of organisms, Edwards et al. (1948) and Moran and Bruner (1949) studied a group of bacteria characterized by Barnes and Cherry (1946) and referred to it as the Bethesda group of bacteria. West and Edwards (1954) found that organisms of both the Bethesda and Ballerup groups were biochemically and serologically indistinguishable and combined the two groups into the Bethesda-Ballerup group. Moreover, West and Edwards (1954) and Møller (1954) called attention to the close biochemical relationship between members of the Bethesda-Ballerup group and strains of *E. freundii*. Accordingly, Kauffmann (1954) reclassified the Bethesda-Ballerup group into *E. freundii*, and later revived the genus *Citrobacter* for *E. freundii* (Kauffmann, 1956).

More recently, Young et al. (1971) described a new genus, *Levinea*, which contained two species, *L. malonatica* and *L. amalonatica*. Ewing and Davis (1972) noted, however, that *L. malonatica* was a later synonym of "*Citrobacter diversum*" which was designated by Werkman and Gillen (1932); consequently, they revived the name *C. diversus* for this species (with a grammatical modification of the ending of the specific epithet). Prior to the work of Young et al. (1971) and Ewing and Davis (1972), Frederiksen (1970) had described a new species, *Citrobacter koseri*. It was confirmed by numerical taxonomy (Sakazaki et al., 1976) and by DNA relatedness (Crosa et al., 1974) that *C. koseri* was also a synonym of *C. diversus*. Although the name *C. diversus* is accepted in the United States, there are many workers in Europe who believe that the original description of "*C. diversum*" by Werkman and Gillen was based on strains which were different from the strains of *C. diversus* described by Ewing and Davis. These workers therefore believe that the name *C. koseri* has priority (Holmes et al., 1974). Because no original strains of "*C. diversum*" exist, it is difficult to judge the dispute

Table 5.13.
Differential characteristics of the genus **Citrobacter** *and biochemically similar genera*[a]

Characteristics	*Citrobacter*	*Salmonella*	*Escherichia*	*Enterobacter*
Lysine decarboxylase	−	+	+	D
Citrate (Simmons')	+	+	−	+
Voges-Proskauer test	−	−	−	+
Growth in KCN medium	D[b]	−	−	+
Indole production	D[c]	−	+	−
Ornithine decarboxylase	D[d]	+	+	+
ONPG hydrolysis[e]	+	D	+	+
Mol% G + C of DNA	50–52	50–53	48–52	52–59

[a] Symbols: +, 90–100% of strains are positive; −, 90–100% of strains are negative; D, different reactions given by different species of a genus.
[b] Only *C. diversus* is negative.
[c] Only *C. freundii* is negative.
[d] Less than 20% of *C. freundii* are negative.
[e] ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

immediately. Thus, the names *C. koseri* and *L. malonatica*, in addition to *C. diversus*, have been included in the Approved Lists of Bacterial Names in 1980.

Ewing and Davis (1972) regarded *L. amalonatica* as a biovar of *C. freundii*. On the basis of results of numerical taxonomy (Sakazaki et al., 1976) and DNA relatedness (Crosa et al., 1974), however, it was obvious that *L. amalonatica* should be placed in a species separate from *C. freundii*, although this organism was more closely related to *C. freundii* and *C. diversus* than to other genera of the family *Enterobacteriaceae*. Thus, Brenner et al. (1977) suggested moving *L. amalonatica* to *Citrobacter*, and the name *L. amalonaticus* has been formally proposed (Brenner and Farmer, 1981, 1982). Macierevicz (1966) studied a group of organisms which were H_2S-negative and ornithine decarboxylase-positive and proposed an illegitimate generic name "*Padlewskia*" without a designation of any specific epithet for the organisms of this genus. From the biochemical characteristics described by Macierewicz, it is clear that *Padlewskia* organisms are identical to *C. amalonaticus*.

"*Citrobacter intermedium*" was proposed by Werkman and Gillen (1932) for H_2S-negative strains of *Citrobacter*. Vaughn and Levine (1942) transferred this species to the genus *Escherichia* as "*E. intermedia*." Sedlák (1974) revived this species in the eighth edition of *Bergey's Manual* as "*C. intermedius*." He described two biovars in *C. intermedius*: biovar "a," corresponding to *L. amalonatica*, and biovar "b," corresponding to *L. malonatica*. It was found, however, that one of Werkman's original strains of *C. intermedius*, ATCC 6750, was a typical *C. freundii* (Frederiksen, 1970). The name *C. intermedius* was, therefore, not included on the Approved Lists of Bacterial Names in 1980 and has no nomenclatural standing.

Differentiation and characteristics of species of **Citrobacter**

The differential characteristics of the species of *Citrobacter* are indicated in Table 5.14. Table 5.15 lists other characteristics of the species.

List of the species of the genus **Citrobacter**

1. **Citrobacter freun'dii** (Braak 1928) Werkman and Gillen 1932, 173,[AL] (*Bacterium freundii* Braak 1928, 140.)

freun'di.i. M.L. gen. n. *freundii* of Freund; named after A. Freund, the bacteriologist who first observed that trimethylene glycol was a product of fermentation.

The morphology is as given for the genus. Usually motile. Usually not encapsulated, although encapsulated strains may occur in some strains belonging to certain O antigen groups.

The colony morphology is similar to that of *Escherichia coli*, but growth may occur on some selective inhibitory media for the isolation of *Salmonella* on which *E. coli* is inhibited.

Physiological and biochemical characteristics are presented in Tables 5.14 and 5.15. Less than 20% of the strains produce ornithine decarboxylase.

Found in man and other animals including mammals, birds, reptiles and amphibians. Also found in soil, water, sewage and food. Often

Table 5.14
Characteristics differentiating **Citrobacter freundii, Citrobacter diversus** *and* **Citrobacter amalonaticus**[a]

Characteristics	1. *C. freundii*	2. *C. diversus*	3. *C. amalonaticus*
Indole production	−	+	+
H_2S production[b]	+	−	−
Arginine dihydrolase	d	+	+
Ornithine decarboxylase	d	+	+
Growth in KCN medium	+	−	+
Malonate utilization	−	+	−
D-Adonitol, acid from	−	+	−
Mol% G + C of DNA	50–51	51–52	51–52

[a] Symbols: +, 90–100% of strains are positive; −, 90–100% of strains are negative; d, different reactions given by different strains of a species.
[b] In Kligler iron agar and triple-sugar iron agar.

Table 5.15.
Other characteristics of **Citrobacter freundii, Citrobacter diversus** *and* **Citrobacter amalonaticus**[a]

Characteristics	1. *C. freundii*	2. *C. diversus*	3. *C. amalonaticus*
Voges-Proskauer test	−	−	−
H_2S production	+	−	−
Urease (Christensen)	d	d	d
Gelatin hydrolysis	−	−	−
Phenylalanine deaminase	−	−	−
d-Tartrate (Kauffmann-Petersen)	(+)	d	−
Mucate, acid from	+	+	+
Esculin hydrolysis	−	d	+
Lipase (Tween 80)	−	−	−
Deoxyribonuclease	−	−	−
Acid from carbohydrates:			
D-Glucose, L-arabinose, cellobiose, maltose, L-rhamnose, trehalose, D-xylose, D-mannitol, D-sorbitol, glycerol	+	+	+
Lactose	d	+	+
Sucrose	d	−	−
Dulcitol	d	d	−
Salicin	−	+	+
Raffinose, erythritol, myo-inositol	−	−	−
Gas from D-glucose	+	+	+
ONPG hydrolysis[b]	+	+	+

[a] Symbols: +, 90–100% of strains are positive; (+), 90–100% of strains are positive after 3 days or more of incubation; −, 90–100% of stains are negative; d, different reactions given by different strains of a species.
[b] ONPG = *o*-nitrophenyl-β-D-galactopyranoside.

found in clinical specimens such as urine, throat, sputum, blood and wound swabs as an opportunistic or secondary pathogen.

The mol% G + C of the DNA is 50–51 (T_m).

Type strain: ATCC 8090.

2. **Citrobacter diversus** (Burkey 1928) Werkman and Gillen 1932, 180.[AL] (*Aerobacter diversum* Burkey 1928, 77; *Citrobacter koseri* Frederiksen, 1970, 93.)

di.ver'sus. L. v. *divertere* to turn in different directions; L. part. adj. *diversus* differing.

The morphology is as given for the genus. Motile. Not encapsulated.

Colonies on nutrient agar are translucent to opaque, resembling those of *Escherichia coli*.

Physiological and biochemical characteristics are presented in Tables 5.14 and 5.15.

Found in the feces of man and other animals and in soil, water, sewage and food. Also isolated from human clinical specimens such as urine, throat, nose and sputum and wound swabs. Occasionally causes neonatal meningitis.

The mol% G + C of the DNA is 51–52 (T_m).

Type strain: ATCC 27156.

3. **Citrobacter amalonaticus** (Young Kenton, Hobbs and Moody 1971) Brenner and Farmer 1982, 266. (*Levinea amalonatica* Young et al. 1971, 58.)

a.ma.lo.na'ti.cus. Gr. prefix *a* not; M.L. adj. *malonaticus* pertaining to malonate; M.L. adj. *amalonaticus* not pertaining to malonate (i.e. not able to utilize malonate).

The morphology is as given for the genus. Motile. Not encapsulated.

Colonies on nutrient agar are translucent to opaque, resembling those of *Escherichia coli*.

Physiological and biochemical characteristics are indicated in Tables 5.14 and 5.15.

Found in the feces of man and other animals and in soil, water and sewage. Also found in a variety of human clinical specimens as an opportunistic pathogen.

The mol% G + C of the DNA is 51–52 (T_m).

Type strain: ATCC 25405.

Genus V. **Klebsiella** *Trevisan 1885, 105*[AL]

IDA ØRSKOV

Kleb.si.el'la. M.L. dim. ending *-ella*; M.L. fem. n. *Klebsiella* named after Edwin Klebs (1834–1913), a German bacteriologist.

Straight rods, 0.3–1.0 μm in diameter and 0.6–6.0 μm in length, arranged singly, in pairs or short chains. Conform to the general definition of the family *Enterobacteriaceae*. **Capsulated.** Gram-negative. **Nonmotile.** Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Grow on meat extract media, producing more or less dome shaped, glistening colonies of varying degrees of stickiness depending on the strain and the composition of the medium. There are no special growth factor requirements. **Oxidase-negative. Most strains can use citrate and glucose as a sole carbon source.** Glucose is fermented with the production of acid and gas (more CO_2 is produced than H_2), but anaerogenic strains occur. Most strains produce 2,3-butanediol as a major end product of glucose fermentation and the **Voges-Proskauer test is usually positive**; lactic, acetic and formic acids are formed in smaller amounts and ethanol in larger amounts than in a mixed acid fermentation. **Fermentation of inositol, hydrolysis of urea, and lack of production of ornithine decarboxylase or H_2S are further distinctive characters.** Some strains fix nitrogen. Occur in intestinal contents, clinical specimens, soil, water, grain, etc. The mol% G + C of the DNA is 53–58 (T_m).

Type species: *Klebsiella pneumoniae* (Schroeter 1886) Trevisan 1887, 94.

Further Descriptive Information

The outermost layer of *Klebsiella* bacteria consists of a large polysaccharide capsule, a character which distinguishes members of this genus from most other bacteria in the family (*Enterobacter aerogenes* and *Escherichia coli* strains with a heat-stable K antigen (A type) may form similar capules). The cell wall itself, however, is structured as that of other *Enterobacteriaceae*, i.e. when going from within: (a) the cytoplasmic membrane, (b) the peptidoglycan layer and (c) the outer membrane containing the lipopolysaccharide (LPS). In addition, *Klebsiella* strains may possess fimbriae (pili), some with a mannose-sensitive adhesin (type 1) and others with a mannose-resistant adhesin (type 3) or with both types (Duguid, 1959).

The production of the large capsules gives rise to large mucoid colonies of a viscid consistency. The capsular material also diffuses freely into the surrounding liquid medium as extracellular capsular material.

Klebsiella strains grow readily on all kinds of media since they have no particular growth requirements. A carbohydrate-rich medium gives a better development of the capsule than a carbohydrate-poor medium. In the author's laboratory a bromothymol blue lactose medium* is most often used.

In general the methyl red test is negative and the Voges-Proskauer (VP) test is positive in *Klebsiella*,, meaning that acetoin and 2,3-butanediol are formed from pyruvic acid and that these neutral end products predominate over the acidic end products as a result of the sugar fermentation. Some strains, e.g. *K. rhinoscleromatis*, do not form acetoin and 2,3-butanediol. Other strains produce acetoin and 2,3-butanediol in such small amounts that the methyl red reaction remains positive. In some strains the acetoin will disappear before the VP reaction is tested. Seemingly paradoxical methyl red and VP reactions may therefore occur (i.e. both tests positive or both tests negative).

Some strains of *Klebsiella* have the ability to fix molecular nitrogen. No particular correlation of this property with the source of a strain seems evident; according to Postgate (1978), "A *Klebsiella* from the gut is as likely—or as unlikely—to fix nitrogen as one from a soil or water sample." Since the nitrogenase is rapidly inactivated in the cells in the presence of oxygen, the nitrogen-fixing ability of *Klebsiella* strains is generally expressed only under anaerobic conditions; however, several reports indicate that low levels of dissolved oxygen (<10 mm Hg) can be tolerated (Klucas, 1972; Hill, 1975; Neilson and Sparell, 1976) or even used to support nitrogen fixation (Hill, 1976). The genetics and regulation of nitrogen fixation in *Klebsiella* have recently been reviewed by Brill (1980).

Klebsiella strains may be lysogenic, but phages used by some workers for phage typing have been isolated from stools or sewage (Slopek et al., 1967; Slopek, 1978).

Many *Klebsiella* strains produce bacteriocin (klebecin) and typing sets of such producers can be selected (Slopek and Maresz-Babczyszyn, 1967; Edmondson and Cook, 1979).

Successful genetic recombinations have been reported in *Klebsiella* (Matsumoto and Tazaki, 1970), and *K. pneumoniae* has been used by several workers for detailed genetic analysis of the genes involved in N_2 fixation (*Nif* genes). These genes are clustered near the *His* region on the chromosome but can be mobilized and transferred to other organisms.

* For composition of bromothymol blue lactose medium, see the genus *Escherichia*.

A high percentage of *Klebsiella* strains from clinical isolates and particularly those from nosocomial infections contain R factors that determine resistance to a variety of drugs, such as β-lactams, cephalosporins, aminoglycosides, tetracyclines, chloramphenicols, sulfonamides and trimethoprim. All *Klebsiella* strains are resistant to ampicillin and this resistance may reside in chromosomal genes or be mediated by genes present on the chromosome and on a plasmid.

In general klebsiellae are good recipients for R factors, a fact that may have made *Klebsiella* a culprit in serious nosocomial epidemic diseases (Falkow, 1975).

Reeve and Braithwaiter (1975) demonstrated two classes of *Klebsiella* strains, one with a strong and the other with a weak lactose-positive phenotype. This was shown to be due to the presence of a *Lac* plasmid in the strongly fermenting strains.

Klebsiella possesses both O (lipopolysaccharide, LPS) and K (polysaccharide) antigens, but serological typing is based on examination of the K antigens. This is because the number of O antigen types is lower than that of the K antigen types and because O antigen determination is hampered by the heat-stable K antigens.

Capsular types A to C of Julianelle (1926) and C to F of W.R.O. Goslings (Onderzoekungen over de bacteriologie en de epidemiologie van het scleroma respiratorium, Thesis, Amsterdam, 1933, pp. 199–201) and of Goslings and Snijders (1936) were redesignated 1 to 6 by Kauffmann (1949) who also established eight new types. Other workers have brought the total number of K types up to 82 (for a review see Ørskov and Ørskov: Serotyping of *Klebsiella*. In Bergan and Norris (Editors), *Methods in Microbiology*, Academic Press, London, in press. The capsular polysaccharides have been analyzed qualitatively (Nimmich, 1968, 1971) and the structures of a majority of them have been determined (for reviews see Heidelberger and Nimmich, 1976; Sutherland, 1977; Rieger-Hug and Stirm, 1981).

The majority of K antigens contain only one charged monosaccharide constituent, most often glucuronic acid, and two to four of the following sugars: galactose, D-glucose, mannose, fucose and L-rhamnose. Other noncarbohydrate constituents, such as acetate or pyruvate, may also be present. For a review of the O antigen structures, see Jann and Jann (1977).

Klebsiellae are opportunistic pathogens that can give rise to bacteremia, pneumonia, urinary tract and several other types of human infection. In recent years there has been an increase in *Klebsiella* infections, particularly in hospitals, due to strains with multiple antibiotic resistance (for a review see Montgomerie, 1979). The gastrointestinal tract is considered to be the main reservoir and the hands of the personnel the main factor for transmission. These outbreaks particularly occur in urological patients and in neonatal and intensive care units. Enterotoxin-producing *Klebsiella* strains have been described (Klipstein et al., 1977).

Klebsiellae are also widely distributed in nature, occurring in soil, water, grain, etc. Many of these environmental strains, however, probably belong to the two newly proposed species, *K. terrigena* and *K. planticola*.

Enrichment and Isolation Procedures

Although klebsiellae are normal inhabitants of the intestinal tract, they are usually present in such low numbers, compared with *E. coli*, that they may be difficult to select after growth for only 24 h; however, they usually will appear as characteristic, elevated, mucoid colonies after incubation for 48 h. The detection and isolation from sources such as feces or water can be facilitated by use of a selective medium. Since *Klebsiella* strains can utilize citrate as a sole carbon source, citrate-containing media have long been used to facilitate their isolation. Methyl violet and double violet agar have been proposed as selective media (Campbell and Roth, 1975; Campbell et al., 1976). A synthetic medium containing *myo*-inositol as the sole carbon source was used successfully for selection of *Klebsiella* (and *Serratia*) by Legakis et al. (1976), and a MacConkey-inositol-carbenicillin agar medium was devised by Bagley and Seidler (1978); the selectivity of the later medium is based upon the high resistance of *Klebsiella* to carbenicillin, in contrast to that of other *Enterobacteriaceae*.

Maintenance Procedures

Klebsiella strains can be easily maintained in meat extract agar stabs or on egg medium when kept at room temperature in the dark. They can be preserved either by storage in broth containing 10% glycerol at −80°C or by lyophilization.

Procedures for Testing Special Characters

Von Riesen (1976) reported that indole-positive strains of *Klebsiella* were able to digest polypectate, and this ability was later shown to be a distinctive character of *K. oxytoca*. The pectate test is negative in the medium of Martin and Ewing (Edwards and Ewing, 1972) but positive in that of Starr (1947). The procedure used by Starr et al. (1967) is as follows. The following ingredients are added to 100 ml of distilled water while stirring: $CaCl_2 \cdot 2H_2O$ (10% solution), 0.6 ml; bromthymol blue (0.1% solution in 6.4×10^{-4} N NaOH), 1.0 ml; yeast extract (Difco), 0.6 g; and sodium polygalacturonate (P-1879, Sigma Chemical, St. Louis, Mo.; or 102921, ICN, Cleveland, Ohio), 3.0 g (added very slowly so that each particle is wetted). After the polygalacturonate is uniformly swelled it is dissolved by bringing the temperature almost to the boiling point with continuous stirring. The pH is adjusted to 7.3 with 1 N NaOH by monitoring the color of the indicator. The medium is sterilized at 121°C for 15 m and dispensed into Petri dishes or tubes. The cultures are either spotted onto or stabbed into the medium, which is then incubated at 30°C and inspected daily for up to 6 days for evidence of liquefaction and/or sinking of the colonies.

The test for liquefaction of gelatin should preferably be the rapid method of Kohn (1953) as described by Lautrop (1956a) and Edwards and Ewing (1972). Most *Klebsiella* strains which liquefy gelatin will do so within 4 days of this method.

Differentiation of the genus **Klebsiella** *from other genera*

See Table 5.3 of the family *Enterobacteriaceae* for characteristics that can be used to distinguish this genus from other genera of the family. The greatest problem is to distinguish *Klebsiella pneumoniae* strains from nonmotile *Enterobacter aerogenes* strains which liquefy gelatin very slowly. The urease test may be of decisive importance in such cases (*K. pneumoniae* is urease-positive).

Taxonomic Comments

In the eighth edition of *Bergey's Manual* three species were described in the genus *Klebsiella*: *K. pneumoniae*, *K. ozaenae* and *K. rhinoscleromatis*. Because DNA reassociation studies have shown that these three species belong to the same DNA relatedness group (Brenner et al., 1972), *K. ozaenae* and *K. rhinoscleromatis* are considered as subspecies of *K. pneumoniae* in the present edition of the *Manual*. Both subspecies may be considered as metabolically inactive biogroups of *K. pneumoniae*: *K. rhinoscleromatis* is the most metabolically inactive, while the metabolic activity in *K. ozaenae* strains is variable. In contrast to *K. pneumoniae* strains (Ewing and Martin, 1974), some *K. ozaenae* strains are arginine dihydrolase-positive. Traditionally, *K. ozaenae* belongs to capsule type 4 (3, 5, 6 or 1/5 have also been described), and if no serotyping is done it is very difficult to distinguish a metabolically active strain of *K. ozaenae* from a strain of *K. pneumoniae*. *K. rhinoscleromatis* may have an as yet undetected phenotypic property, since it is found constantly and exclusively in patients with rhinoscleroma as well as in their contacts. Three subspecies of *K. pneumoniae* are proposed: *K. pneumoniae* subsp. *pneumoniae* (subsp. nov.), *K. pneumoniae* subsp. *ozaenae* (subsp. nov.), and *K. pneumoniae* subsp. *rhinoscleromatis* (subsp. nov.). The description of each of the three subspe-

cies corresponds to that of the former species. It is recommended that clinical laboratories omit these subspecies designations for routine reporting.

Indole-positive and gelatin-liquefying strains of *Klebsiella* have been a taxonomic problem for years. Some authors have considered them as biogroups of *K. pneumoniae* (Edwards and Ewing, 1975; Ørskov, 1974); others as a separate group (Lautrop, 1956b; Stenzel et al., 1972), and still others have excluded such strains from their studies of *Klebsiella*. The name *Bacterium oxytocus* Flügge was revived by Lautrop (1956b) for these strains, and the name *Aerobacter oxytocum* was recognized in earlier editions of *Bergey's Manual*. Korth et al. (1960) showed that the "oxytoca" variants of *Klebsiella* produce a dark brown pigment when grown on a defined medium containing gluconate and ferric citrate. On the basis of DNA/DNA hybridization studies, it has been proposed that indole- and gelatin-positive strains be removed from the genus *Klebsiella* (Jain et al., 1974) or that they be considered as a separate *Klebsiella* species designated *K. oxytoca* (Brenner et al., 1977). The latter course has been followed in the present edition of the *Manual*.

A third proposed species of *Klebsiella* is *K. terrigena* (Izard et al., 1981), a name recently coined for strains which are derived mainly from aquatic and soil environments (Izard et al., 1981). According to numerical taxonomic analysis (Gavani et al., 1977; Naemura et al., 1979) and DNA/DNA hybridization studies (Woodward et al., 1979; Izard et al., 1981), *K. terrigena* forms a species distinct from both *K. pneumoniae* and *K. oxytoca*. Phenotypically, *K. terrigena* is closely related to *K. pneumoniae*, but three tests (growth at 10°C (positive for *K. terrigena*), gas production from lactose when incubated at 44.5°C (negative for *K. terrigena*), and fermentation of melizitose (positive for *K. terrigena*)) can differentiate the two species.

A fourth proposed species of *Klebsiella* is *K. planticola* (Bagley et al., 1981), which contains strains isolated primarily from botanical and soil environments (Bagley et al., 1981). *K. planticola* is distinct from other *Klebsiella* species on the basis of numerical taxonomy (Gavini et al., 1977; Naemura et al., 1979) and by DNA relatedness (Woodward et al., 1979; Izard et al., 1981). Like *K. terrigena*, *K. planticola* can be separated from *K. pneumoniae* by growth at 10°C (positive for *K. planticola*) and by gas production from lactose when incubated at 44.5°C (negative for *K. planticola*). Melizitose is fermented by *K. terrigena* but not by *K. pneumoniae* or *K. planticola*.

In this edition of the *Manual*, the genus *Klebsiella* is confined to nonmotile strains. Proposals have been made to transfer *Enterobacter aerogenes* to the genus *Klebsiella* as *K. mobilis* (Bascomb et al., 1971; Izard et al., 1980). *E. aerogenes* is biochemically and genetically as related or more related to klebsiellae than to most other *Enterobacter* species (Bascomb et al., 1971; Brenner et al., 1972; Steigerwalt et al., 1975; Izard et al., 1980). If transferred to the genus *Klebsiella*, *E. aerogenes* would normally become the new combination "*K. aerogenes*," a name that has no standing in nomenclature. It had been used to designate certain strains of *K. pneumoniae* and, therefore, should not be reproposed for a different group. The proposal of *K. mobilis* poses several problems: (a) a Judicial Commission decision would be required to change the specific epithet from *aerogenes* to *mobilis*; (b) the well accepted epithet *aerogenes* would be lost; (c) the epithet *mobilis* is misleading because not all strains of *E. aerogenes* are motile; (d) the important genus characteristic, lack of motility, would no longer be definitive for the genus *Klebsiella*.

Further Comments

Cowan et al. (1960) recognized five species in the *Klebsiella* group: *K. aerogenes*, *K. pneumoniae* (*sensu stricto*), *K. ozaenae*, *K. rhinoscleromatis*, and *K. edwardsii* with two varieties: *K. edwardsii* var. *edwardsii* and *K. edwardsii* var. *atlantae*. Durlakowa et al. (1967) and Slopek and Durlakowa (1967) divided *Klebsiella* into the six taxa of Cowan et al.; the names and the rank were, however, somewhat changed. Bascomb et al. (1971) divided *Klebsiella* into six taxa, one of which was *K. pneumoniae* (*sensu stricto*) and another composed of *K. aerogenes*, *K. edwardsii* and indole-forming *Klebsiella* strains. Brenner et al. (1972) found 80–90% DNA relatedness between *K. pneumoniae* (*sensu lato*), *K. ozaenae*, *K. rhinoscleromatis* and *K. edwardsii*. No *K. pneumoniae* (*sensu stricto*, according to Cowan et al., 1960, or Bascomb et al., 1971) was included in that study. However, the neotype strain of *K. pneumoniae*, ATCC 13883 (Ørskov, 1974), which is a *K. pneumoniae* (*sensu stricto*) strain (VP-negative, KCN-negative), has been shown to be genetically indistinguishable from other *Klebsiella pneumoniae* (*sensu lato*) strains (Seidler et al., 1975; Woodward et al., 1979). The classification of Cowan et al. (1960) is used in the United Kingdom and at other places, but never in the United States. This means that the same organism will be classified either as *K. pneumoniae* or *K. aerogenes*, depending on the country.

The existence of two additional *Klebsiella* species that contain strains of environmental origin is suggested in the studies by Naemura et al. (1979) and Woodward et al. (1979).

Differentiation and characteristics of the species of the genus **Klebsiella**

Table 5.16 presents the characteristics differentiating the four species of *Klebsiella*, and Table 5.17 lists additional characteristics of the species. Table 5.18 lists those characteristics that differentiate the three subspecies of *K. pneumoniae*.

List of the species of the genus **Klebsiella**

1. **Klebsiella pneumoniae** (Schroeter 1886) Trevisan 1887, 94.[AL] (Includes *Aerobacter aerogenes* as described in the seventh edition of *Bergey's Manual* (Breed, 1957). (*Hyalococcus pneumoniae* Schroeter 1886, 1952.)

pneu.mo'ni.ae. Gr. n. *pneumonia* pneumonia, inflammation of the lungs; M.L. gen. n. *pneumoniae* of pneumonia.

The characteristics are as described for the genus and as listed in Tables 5.16 to 5.18.

K. pneumoniae can be divided into many biovars (Ørskov, 1957; Rennie and Duncan, 1974).

K. pneumoniae is normally found in the intestinal tract of man and animals, but in low numbers compared with *E. coli*. It may be isolated in association with several pathological processes in man, e.g. infection of the urinary and respiratory tracts. Capsule types 1, 2 and 3 may be the causative agent of pneumonia. In animals, *K. pneumoniae* may be isolated from metritis in mares and bovine mastitis.

The mol% G + C of the DNA is 56–58 (T_m) (Seidler et al., 1975). The intraspecies DNA relative reassociation values is ~80–90% (Brenner et al., 1972) or 73–100% (Woodward et al., 1979).

Type strain: ATCC 13883 (NCTC 9633; CDC 298-56).

1a. **Klebsiella pneumoniae** subspecies **pneumoniae** (Schroeter 1886) Trevisan 1887, 94.[AL]

Distinguished from the subspecies *ozaenae* and *rhinoscleromatis* by the characteristics listed in Table 5.18.

Type strain: ATCC 13883.

1b. **Klebsiella pneumoniae** subspecies **ozaenae** subsp. nov. (*Klebsiella ozaenae* (Abel 1893) Bergey, Harrison, Breed, Hammer and Huntoon 1925, 266; *Bacillus mucosus ozaenae* Abel 1893, 167; *Bacillus ozaenae* (Abel 1893) Lehmann and Neumann 1896, 204.)

o.zae'nae. L. fem. n. *ozaena* ozena; L. gen. n. *ozaenae* of ozena.

Table 5.16.
Differential characteristics of the species of the genus **Klebsiella**[a]

Characteristics	1. *K. pneumoniae*	2. *K. oxytoca*	3. *K. terrigena*	4. *K. planticola*
Indole production	−	+	−	d
Pectate degradation	−	+	−	−
Fecal coliform test (gas production from lactose at 44.5°C)	+	−	−	−
Growth at 10°C	−	+	+	+
Fermentation of:				
Inulin	−	+	d	d
D-Melizitose	−	d	+	−
L-Sorbose	d	+	+	+
Utilization of:				
Gentisate or *m*-hydroxybenzoate	−	+	+	−
Hydroxy-L-proline	d	d	d	+

[a] Symbols: see standard definitions.

Table 5.17.
Other characteristics of the species of the genus **Klebsiella**[a]

Characteristics	1. *K. pneumoniae*	2. *K. oxytoca*	3. *K. terrigena*	4. *K. planticola*
Methyl red test	−	−	+	d
Voges-Proskauer test	+	+	+	+
Fermentation of:				
L-Arabinose, *myo*-inositol, lactose, D-mannitol, L-rhamnose, sucrose, D-glucose, raffinose, D-sorbitol	+	+	+	+
Adonitol	d	+	+	d
Dulcitol	d	d	−	d
Utilization of:				
Citrate (Simmons')	+	+	+	+
Malonate	+	d	d	+
Utilization of organic acids:				
Sodium citrate	d	+		
d-Tartrate	d	+	+	d
Arginine dihydrolase (Møller)	−	−	−	−
Lysine decarboxylase (Møller)	+	+	+	+
Ornithine decarboxylase (Møller)	−	−	−	−
Gelatin hydrolysis	−	d	−	−
H_2S production (triple-sugar iron agar)	−	−	−	−
Urease	+	+	+	+
Formation of 2-ketogluconate from gluconate	−	d	−	−

[a] For symbols see standard definitions.

Distinguished from the subspecies *pneumoniae* and *rhinoscleromatis* by the characteristics listed in Table 5.18.

Occurs in ozena and other chronic diseases of the respiratory tract.

Type strain: ATCC 11296 (NCTC 5050).

Table 5.18.
Differential characteristics of the subspecies of **Klebsiella pneumoniae**[a]

Characteristics	1a. *pneumoniae*	1b. *ozaenae*	1c. *rhinoscleromatis*
Gas from glucose	+	d	+
Acid from:			
Lactose	+	(+)	−
Dulcitol	d	−	−
Methyl red test	−	+	+
Voges-Proskauer test	+	−	−
Utilization of:			
Citrate (Simmons')	+	d	−
Malonate	+	−	+
Urease	+	d	−
Utilization of organic acids (Kauffmann-Petersen):			
Citrate	d	d	−
d-Tartrate	d	d	−
Mucate	+	d	−
Lysine decarboxylase (Møller)	+	d	−
Arginine dihydrolase (Møller)	−	d	−

[a] For symbols see standard defintions; also (+), slow fermentation.

1c. **Klebsiella pneumoniae** subspecies **rhinoscleromatis** subsp. nov. (*Klebsiella rhinoscleromatis* Trevisan 1887, 95; *Bacterium rhinoscleromatis* (Trevisan 1887) Migula 1900, 352.)

rhi.no.scle.ro'ma.tis. M.L. adj. *rhinoscleromatis* pertaining to rhinoscleroma.

Distinguished from the subspecies *pneumoniae* and *ozaenae* by the characteristics listed in Table 5.18.

Found in patients with rhinoscleroma.

Type strain: ATCC 13884 (NCTC 5046).

2. **Klebsiella oxytoca** (Flügge 1886) Lautrop 1956, 375.[AL] (*Bacillus oxytocus perniciosus* Flügge 1886, 268.)

ox.y.to'ca. Gr. *oxys* sour, acid; Gr. suffix *-tokos* bearer, producer; M.L. n. *oxytocus* acid-producer; spurious M.L. adj. *oxytoca* (*sic*) acid-producing.

The characteristics are as described for the genus and as listed in Tables 5.16 and 5.17.

Present in the intestinal tract of man and animals. Can be isolated from various pathological processes and also from botanical and aquatic environments.

K. oxytoca strains are encapsulated. Some of the K antigen test strains are *K. oxytoca*; however, in very few, if any, cases has a particular kind of K antigen been found only in *K. oxytoca* strains.

The mol% G + C of the DNA ranges from 55–58 (T_m). The intraspecies DNA relative reassociation values was 75% in the study by Brenner et al. (1975) and 95% (average value) in the study by Woodward et al. (1979).

Type strain: ATCC 13182.

3. **Klebsiella terrigena** Izard, Ferragut, Gavini, Kersters, De Ley and Leclerc 1981, 116.[VP]

ter.ri.ge'na. L. n. *terra* soil; L. suffix *gena* origin; M.L. n. *terrigena* from soil.

The characteristics are as described for the genus and as listed in Tables 5.16 and 5.17.

Isolated mainly from aquatic and soil environments.

Phenotypically, *K. terrigena* resembles *K. pneumoniae*; however, it can be distinguished by its ability to grow at 10°C, its inability to produce gas from lactose at 44.5°C, and by its ability to ferment melizitose.

The mol% G + C of the type strain was 56.7 (T_m) (Izard et al., 1981). The average intraspecies DNA relative reassociation value is above 86% (Izard et al., 1981).

Type strain: CIP 80-07 (CUETM 77-176; Gavini et al. L 84).

4. **Klebsiella planticola** Bagley, Seidler and Brenner 1982, 266.[VP*] (Effective publication: Bagley et al. 1981, 105.)

plan.ti'co.la. L. fem. n. *planta* a plant; L. suff. *-cola* dweller; M.L. fem. n. *planticola* plant-dweller.

The characteristics are as described for the genus and as listed in Tables 5.16 and 5.17.

Isolated mainly from botanical, aquatic and soil environments.

Three biovars have been described (Naemura et al., 1979).

K. planticola can be distinguished from *K. pneumoniae* by its ability to grow at 10°C and by its inability to produce gas from lactose at 44.5°C. Its inability to ferment melizitose distinguishes *K. planticola* from *K. terrigena*.

Encapsulated; typable with *Klebsiella* K antisera.

The mol% G + C of the two strains tested was 53.9 and 55.4 (T_m) (Seidler et al., 1975). The average intraspecies DNA relative reassociation value is above 75% (Woodward et al., 1979).

Type strain: ATCC 33531 (V-236; CDC 4245-72).

Genus VI. **Enterobacter** *Hormaeche and Edwards 1960, 72[AL]; Nom. Cons. Opin. 28, Jud. Comm. 1963, 38*

C. RICHARD

En.te.ro.bac'ter. Gr. neut. n. *enteron* intestine; M.L. masc. n. *bacter* equivalent of bacterium, a small rod; M.L. masc. n. *Enterobacter* intestinal small rod.

Straight rods, 0.6–1.0 μm wide × 1.2–3.0 μm long, conforming to the general definition of the family *Enterobacteriaceae*. Gram-negative. **Motile by peritrichous flagella** (generally 4–6). Facultatively anaerobic. Grow readily on ordinary media. Ferment glucose with production of acid and gas (generally CO_2:H_2 = 2:1). Gas is not produced from glucose at 44.5°C. Most strains give a **positive Voges-Proskauer reaction** and a negative methyl red test. Citrate and malonate are usually utilized as sole sources of carbon and energy. Hydrogen sulfide is not produced from thiosulfate. Gelatin is liquefied slowly by most strains. **Deoxyribonuclease (DNase), Tween 80 esterase and lipase are not produced.** Optimum temperature for growth, 30°C. Most clinical strains grow at 37°C; some environmental strains give erratic biochemical reactions at 37°C. Widely distributed in nature; common in man and animals. The mol% G + C of the DNA is 52–60 (Bd).

Type species: *Enterobacter cloacae* (Jordan 1890) Hormaeche and Edwards 1960, 72.

Further Descriptive Information

The genus *Enterobacter* belongs to group II of the family *Enterobacteriaceae* as indicated in the eighth edition of the *Manual* and is therefore characterized by a positive Voges-Proskauer (VP) reaction and β-galactosidase (ONPG test). Unlike the genus *Klebsiella*, *Enterobacter* is motile, and unlike the genus *Serratia*, *Enterobacter* is negative for lipase, Tween 80 esterase and DNase.

Enterobacter species grow rapidly on the usual enteric media. In general, strains from environmental sources grow better at 20–30°C rather than 37°C; whereas strains from clinical sources grow better at 37°C. On Drigalski lactose agar, *E. cloacae* forms colonies that are lactose-positive or negative, round (2–3 mm in diameter), and slightly iridescent or flat with irregular edges. On Hektoen medium, colonies have a similar diameter and are salmon-pink colored. On eosin methylene blue agar the colonies are pinkish, mucoid and convex, 3–4 mm in diameter. *E. sakazakii* grows rapidly on nutrient agar or tryptic soy agar, forming bright yellow colonies at 25°C or pale yellow colonies at 37°C, 1–3 mm in diameter. Various colony types of *E. sakazakii* are observed: typical smooth colonies, mucoid rubbery colonies, and occasionally dry colonies (Farmer et al., 1980). Aerogenic strains of *E. agglomerans* form colonies resembling those of *E. cloacae*, whereas anaerogenic strains, especially those of biogroup 1, may present different morphologies: (a) rough and wrinkled colonies that are rather difficult to remove with a platinum wire, (b) smooth, irregularly round colonies, (c) "cauliflower" rough colonies, and (d) convex mucoid colonies (particularly on media containing carbohydrates). Anaerogenic strains often elaborate a yellow pigment (75% of all strains, 85% of biogroup 1 strains), whereas this is less common with aerogenic biogroup strains (less than 50%) (Richard, 1978). Low temperatures (20–30°C) are better for pigment production than 37°C. The carotenoid-like yellow pigment is soluble in ethanol and acetone but is insoluble in water and chloroform. Colonies of *E. aerogenes* resemble those of *E. cloacae*. The colonial morphology occurring on media containing methyl violet as a selective agent can be used to differentiate *E. aerogenes* and other Gram-negative organisms from *Klebsiella pneumoniae* (Campbell and Roth, 1975). *E. gergoviae* colonies resemble those of *E. cloacae* and *E. aerogenes*.

Biochemical reactions differ widely among the species and biogroups of *Enterobacter*, and carbohydrate fermentation tests and amino acid decarboxylase tests are useful for differentiation. *E. cloacae* produces acid and gas rapidly from cellobiose and produces acid slowly from glycerol. Although lactose may be fermented slowly, *E. cloacae* is always positive for β-galactosidase (ONPG test). Some strains of *E. cloacae* utilize malonate and ferment adonitol. The enzyme β-xylosidase is present. *E. cloacae* is positive for arginine dihydrolase (ADH) and ornithine decarboxylase (ODC) but is negative for lysine decarboxylase (LDC). Most strains liquefy gelatin slowly. Some strains of *E. cloacae* (and *E. aerogenes*) have the ability to fix molecular nitrogen under anaerobic conditions (e.g., Neilson and Sparell, 1976; Nelson et al., 1976).

E. sakazakii shows biochemical characters similar to those of *E. cloacae*, but does not ferment D-sorbitol or mucate and has a delayed DNase reaction.

E. agglomerans is negative for ADH, ODC and LDC.

E. aerogenes is motile, positive for ODC, negative for urease, and can utilize *m*-hydroxybenzoate as a sole carbon and energy source. These are useful tests for distinguishing this species from *K. pneumoniae*.

E. gergoviae does not ferment D-sorbitol or mucate, is negative for β-xylosidase and gelatinase, and is positive for ODC and LDC but negative for ADH. *E. gergoviae* is urease-positive, whereas other *Enterobacter* species are urease-negative.

E. intermedium (Izard et al., 1980a) and *E. amnigenus* (Izard et al., 1981) are new species on the basis of DNA relatedness to one another and to other *Enterobacter* species. They can be separated from other *Enterobacter* species by their inability to grow at 41°C and by the reactions given in Table 5.20.

Biotyping, sometimes serotyping (by use of O and H antigens and occasionally capsular antigens) and antibiotic susceptibility may be used as epidemiological markers for *Enterobacter* strains. With regard to antigenic characters, 53 O antigens, 56 H antigens and 79 different serovars have been described for *E. cloacae* (Sakazaki and Namioka, 1960). The fermentation of various carbohydrates (adonitol, lactose, mucate, L-rhamnose, dulcitol, salicin, sucrose, α-methylglucoside, glycerol), malonate utilization, and the presence of β-galactosidase, β-

* *VP* denotes that this name has been validly published in the official publication, International Journal of Systematic Bacteriology.

xylosidase, gelatinase, ODC and ADH may be used as markers for epidemiological studies of *E. cloacae*. With regard to *E. aerogenes*, ~80% of the strains possess a thin capsule that is antigenically related to the capsular antigens of *Klebsiella* (chiefly antigens K68 and K26, and occasionally antigens K4, K11, K42 and K59). *E. aerogenes* and *Klebsiella* antigens are not identical but do have common fractions which are responsible for the cross-reactions (Richard, 1977).

Concerning antibiotic susceptibility, most *Enterobacter* strains are resistant to ampicillin and cephalosporins (Toala et al., 1970), but are generally sensitive to carbenicillin and the newer cephalosporins, such as cefotaxime (Sirot et al., 1980). Some strains of *E. cloacae* and *E. aerogenes* found in hospitals are resistant to tetracycline, aminoglycosides and sulfonamides.

E. cloacae is the most frequently isolated *Enterobacter* species from man and animals. It is found in human and animal feces, but is not known to be an enteric pathogen. It is, however, an opportunistic pathogen isolated from urine, sputum and the respiratory tract, pus, and occasionally from blood or spinal fluid. It has an increasing importance in hospitals, especially in intensive care units, emergency units and urology.

E. sakazakii is often a commensal without clinical significance and is occasionally a pathogen causing neonatal meningitis and bacteremia.

E. agglomerans can behave as an opportunistic pathogen in immunologically compromised patients such as neonates, premature infants, burned or multiply traumatized patients, and patients with leukemia or who are undergoing immunosuppressive therapy. Strains of *E. agglomerans* are frequently isolated by blood culture because they are generally introduced by such invasive procedures as catheterization, intubation, and surgical or medical acts. Such contaminations result in a transitory bacteremia and occasionally septicemia (Richard, 1978).

E. aerogenes is found in human and animal feces, but is not known to be an enteric pathogen. It is an opportunistic pathogen and is isolated from the respiratory tract, genitourinary tract, pus, and occasionally from blood and spinal fluid. Like *Klebsiella pneumoniae*, it appears to be a normal constituent of the preputial flora of healthy stallions and, therefore, may be an etiologic agent of epidemic metritis in mares (Plate and Atherton, 1976).

E. gergoviae sometimes appears to be an opportunistic pathogen and has been isolated from urine, pus, sputum, blood and other clinical specimens. The species has been implicated in a long term nosocomial outbreak of urinary tract infections (Richard et al., 1976).

E. amnigenus and *E. intermedium* have not been isolated from human infection.

All *Enterobacter* species are found in the natural environment (water, sewage, soil, vegetables), especially *E. agglomerans*—called *Erwinia herbicola* by phytopathologists. *E. agglomerans* is a saprophytic microorganism frequently isolated from plants, flowers, seeds and vegetables (it is probably not phytopathogenic) and from a wide variety of environmental sources such as water, soil and foodstuffs. *E. cloacae* is found in water, sewage, soil and meat. *E. sakazakii* is rarely encountered in clinical specimens and is more prevalent in the environment and in food. *E. aerogenes* is found in water, sewage, soil and dairy products. *E. gergoviae* has been isolated from various environmental sources (cosmetics, water, etc.). *E. amnigenus* and *E. intermedium* are found in drinking and surface water and in unpolluted soil.

Enrichment and Isolation Procedures

All media designed for the isolation of *Enterobacteriaceae* can be used for the isolation of *Enterobacter* species: MacConkey agar, Drigalski lactose agar, Hektoen agar, deoxycholate lactose citrate agar, etc. *Enterobacter* can also grow on media for general use, such as blood agar, nutrient agar, tryptic soy agar, bromocresol purple lactose agar, etc.

Media specifically selective for *Enterobacter* are not available.

Maintenance Procedures

Strains are initially grown on tryptic soy agar at their optimum temperature. They are then inoculated by stabbing a maintenance medium* designed for maintenance of *Enterobacteriaceae* and related organisms. The cultures are then stored at room temperature in a dark, dry place.

Cultures may be also preserved by freeze-drying. Freeze-drying is the best procedure for preservation of pigmented strains.

Differentiation of the genus **Enterobacter** *from other genera*

Table 5.19 provides the main characteristics that can be used to differentiate the genus *Enterobacter* from the genera *Klebsiella*, *Hafnia* and *Serratia*.

Taxonomic Comments

Enterobacter cloacae is the type species of the genus *Enterobacter*. Strains of *E. sakazakii* were previously called yellow-pigmented *E. cloacae*; however, DNA/DNA hybridization studies have shown that *E. cloacae* strains form one DNA relatedness group different from that containing the yellow strains (which are now named *E. sakazakii*) (Steigerwalt et al., 1976). The type strain of *E. sakazakii* is 83–89% related to other *E. sakazakii* strains and only 31–54% related to nonpigmented *E. cloacae* strains (Farmer et al., 1980).

E. agglomerans is a heterogeneous species that is synonymous with *Erwinia herbicola*, *Erwinia uredovora* and *Erwinia stewartii*. Ewing and Fife (1972) proposed that the strains from clinical sources be designated as *Enterobacter agglomerans* because the characteristics of the organisms were in conformity with the genus *Enterobacter*. On the other hand, strains of interest to phytopathologists have been placed in the genus *Erwinia* (see that article on *Erwinia* in this *Manual*). It currently is difficult, if not impossible, to distinguish strains from different sources due to the diversity in this group of organisms. Further phenotypic and genotypic studies must be done to define the groups now referred to as *E. agglomerans* and *Erwinia* species. See the article on the family *Enterobacteriaceae* in this *Manual* for further information concerning this problem.

E. aerogenes and *Klebsiella pneumoniae* have a number of characteristics in common; however, it has been shown that they represent two distinct DNA/DNA homology groups. Only 56% relatedness is observed between *Klebsiella* and *E. aerogenes*, whereas *E. cloacae* exhibits 40% relatedness with *K. pneumoniae* and *E. aerogenes* (Brenner et al., 1972). Because *E. aerogenes* does exhibit some phenotypic and genetic similarity to *K. pneumoniae*, some bacteriologists have proposed the transfer of *E. aerogenes* into the genus *Klebsiella* as *K. mobilis* (Bascomb et al., 1971; Izard et al., 1980).

E. gergoviae is a urease-positive *Enterobacter* species which shares many characters with *E. aerogenes*. The biochemical homogeneity within *E. gergoviae* is reflected by a high level of genetic relatedness among strains from France, the United States and Africa (relative binding ratio at 60°C, 76–97%) (Brenner et al., 1980).

E. hafniae has been transferred previously to the genus *Hafnia* as *Hafnia alvei* because it has few phenotypic or genetic similarities with other *Enterobacter* species.

E. liquefaciens has been transferred to the genus *Serratia* as *S. liquefaciens* because it is closely related to *S. marcescens* by biochemical and genetic properties.

* Maintenance medium (g/liter): Bacto-peptone (Difco), 10.0; NaCl, 5.0; Bacto-agar (Difco), 10.0; pH 7.4. The medium should be dispensed into small (9.5–10 × 90 mm) screw-capped tubes.

Enterobacter intermedium and *Enterobacter amnigenus* (see "Other Organisms Belonging to the Genus Enterobacter") are phenotypically closest to *E. cloacae* but are distinct by DNA/DNA hybridization (Izard et al., 1980a; Izard et al., 1981).

Atypical strains that are difficult to assign to the described species of the genus *Enterobacter* are occasionally encountered.

Differential characteristics of **Enterobacter** *species*

Tables 5.20, 5.21 and 5.22 give characteristics useful for the differentiation of the various species of *Enterobacter*.

List of the species of the genus **Enterobacter**

1. **Enterobacter cloacae** (Jordan 1890) Hormaeche and Edwards 1960, 72;[AL] Nom. Cons., Opinion 28, Jud. Comm. 1963, 38. (*Bacillus cloacae* Jordan 1890, 836.)

clo.a'cae. L. n. *cloaca* a sewer; L. gen. n. *cloacae* of sewer.

The characteristics are described in Tables 5.20, 5.21 and 5.22. The following tests should be emphasized for identification of the species: LDC-negative, ODC- and ADH-positive.

E. cloacae has a natural resistance to ampicillin. Many strains are resistant to cephalosporins, chloramphenicol, tetracycline and sulfonamides. Most strains are sensitive to aminoglycosides (except streptomycin), colistin, nalidixic acid and nitrofuranes.

E. cloacae is less susceptible to chlorination than *Escherichia coli*.

Occurs in water, sewage, soil, meat, hospital environments and on the skin and in the intestinal tracts of man and animals as a commensal.

The mol% G + C of the DNA is 52–54 (T_m).

Type strain: ATCC 13047 (NCTC 10005, CDC 279-56).

Table 5.19
Differentiation between **Enterobacter** *and related genera*[a]

Characteristics	*Entero-bacter*	*Klebsiella*	*Hafnia*	*Serratia*
Motility	+	−	+	+
Ornithine decarboxylase	+	−	+	[+]
Arginine dihydrolase	D	−	−	−
Deoxyribonuclease	−	−	−	+
Gelatinase	D	D	−	[+]
Citrate utilization	[+]	[+]	−	+
Susceptible to *Hafnia* phage[b]	−	−	+	−
D-Sorbitol (acid)	[+]	+	−	[+]

[a] Symbols: +, all strains positive in 24–48 h; [+], majority of strains positive (generally more than 89%); −, all strains negative after 7 days D, differs among species.
[b] Guinée and Valkenburg, 1968.

2. **Enterobacter sakazakii** Farmer, Asbury, Hickman and Brenner 1980, 575.[VP]

sa.ka.za'ki.i. M.L. gen. n. *sakazakii* of Sakazaki; named after the Japanese bacteriologist Riichi Sakazaki.

Previously known as "yellow-pigmented *E. cloacae*."

The characteristics are as described in Tables 5.20 and 5.22. The biochemical characteristics are similar to those of *E. cloacae*, but *E. sakazakii* does not ferment D-sorbitol and mucate and gives a delayed positive DNase test. The nondiffusible yellow pigment (best formed at 25°C) is useful for identification; this pigment may be lost upon subculturing. Approximately 10% of the strains produce indole. Table 5.22 presents the main characters useful for differentiating *E. sakazakii*, *E. agglomerans* and *E. cloacae*.

Generally susceptible to ampicillin, carbenicillin, aminoglycosides, chloramphenicol, tetracycline and nalidixic acid; 87% of the strains are resistant to cephalothin.

Occurs in the environment and in foods, rarely in clinical specimens.

The mol% G + C of the DNA is 57 (T_m).

Type strain: ATCC 29544 (CDC 4562-70).

Table 5.20.
Differential characteristics of the species of the genus **Enterobacter** *and of* **Hafnia alvei**[a]

Characteristics	1. *E. cloacae*	2. *E. sakazakii*	3. *E. agglomerans*	4. *E. aerogenes*	5. *E. gergoviae*	a. *E. inter-medium*	b. *E. amni-genus*	*Hafnia al-vei*
KCN	+	+	d	+	−	d	d	+
Urease	−	−	−	−	+	−	−	−
Gelatinase	(+)	[+]	[+]	d	−	−	−	−
Decarboxylases:								
Lysine	−	−	−	+	+/(+)	−	−	+
Ornithine	+	+	−	+	+	+	+	+
Arginine	+	+	−	−	−	−	+	−
β-Xylosidase[b]	+	+	d	+	−	+	+	−
Acid from:								
Sorbitol	+	−	d	+	−	+	d	−
Sucrose	+	+	d	+	+	d	d	−
Raffinose	[+]	+	d	+	+	+	+	−
α-Methylglucoside	+	+	d	+	−	+	d	−
Mucate	d	−	d	+	−	+	+	−
Citrate (Simmons')	+	+	[+]	+	+	+	+	−
Indole	−	d	d	−	−	−	−	−
Yellow pigment formed	−	+	d	−	−	−	−	−

[a] Symbols: +, all strains positive in 24–48 h; [+], majority of strains positive (generally more than 89%); (+), delayed positive (positive between 3 and 7 days); −, all strains negative after 7 days; +/(+), some strains positive in 24–48 h, some strains positive between 3 and 7 days; d, differs among strains (generally between 11 and 80% positive).
[b] From Brisou et al. (1972).

Table 5.21.
Biochemical characteristics of **Enterobacter agglomerans** *and* **Enterobacter cloacae**[a]

Characteristics	1. *E. cloacae*	3. *E. agglomerans*	
		Anaerogenic strains	Aerogenic strains
Yellow pigment	−	[+]	d
Gas from D-glucose	+	−	+
Nitrate reductase	+	[+]	+
Indole	−	[−]	[−]
Voges-Proskauer	+	[+]	d
Decarboxylases:			
Ornithine	+	−	[−]
Arginine	+	−	−
Lysine	−	−	−
KCN	+	[−]	[+]
Methyl red	−	[+]	[+]
D-Sorbitol, acid	+	[−]	[+]
Raffinose, acid	+	[−]	d
Mucate, acid	[+]	[−]	d
β-Xylosidase	+	[−]	[+]
Gelatinase	+	[+]	d
Motility	+	[+]	[+]
Acid from:			
D-Xylose, L-arabinose, D-mannitol	+	+	+
D-Adonitol	d	[−]	[−]
L-Rhamnose, maltose	+	[+]	+
Sorbose, D-tartrate	−	−	−
Lactose	[+]	[−]	d
Sucrose	+	d	d
myo-Inositol	[−]	[−]	[−]
Salicin	[+]	d	d
Cellobiose	+	d	+
Glycerol	d	d	d
Melibiose	[+]	[−]	[+]
β-Galactosidase (ONPG test)	+	+	+
Citrate:			
Simmons'	+	[+]	[+]
Christensen's	+	+	+
Tetrathionate reductase (TTR)	−	−	−
Malonate	[+]	d	[+]
Urease, H_2S	−	−	−
Phenylalanine and tryptophan deaminase	−	−	−

[a] Symbols: +, all strains positive in 24–48 h; [+], majority of strains positive (generally more than 89%); [−], majority of strains negative (generally more than 89%) after 7 days; −, all strains negative after 7 days; d, differs among strains (generally from 11–89% positive).

3. **Enterobacter agglomerans** (Beijerinck 1888) Ewing and Fife 1972, 10.[AL] (*Bacillus agglomerans* Beijerinck 1888, 749; *Erwinia herbicola* (Geilinger 1921) Dye 1964, 268; *Bacterium herbicola* Geilinger 1921, 105; *Erwinia uredovora* (Pon, Townsend, Wessman, Schmitt and Kingsolver 1954) Dye 1963, 149; *Xanthomonas uredovorus* Pon, Townsend, Wessman, Schmitt and Kingsolver 1954, 710: *Erwinia stewartii* (Smith 1898) Dye 1963, 504; *Pseudomonas stewartii* Smith 1898, 422; *Escherichia adecarboxylata* Leclerc 1962, 736.)

ag.glo'mer.ans. L. v. *Agglomerare* to form into a ball; L. part. adj. *agglomerans* forming into a ball (referring to the occurrence of the bacteria in aggregates surrounded by a translucent sheath (symplasmata) in anaerogenic strains).

The biochemical characteristics of *E. agglomerans* are as described in Tables 5.20, 5.21 and 5.22. Tables 5.23 and 5.24 present the biochemical characters of the 11 biogroups (7 anaerogenic and 4 aerogenic). The majority of strains are anaerogenic (80%: and Ewing and Fife, 1972; 62%: Richard, 1975, 1978).

The biochemical characters of *E. cloacae* and *E. agglomerans* (aerogenic and anaerogenic groups) are compared in Table 5.21. the aerogenic biogroups are closely related to *E. cloacae*, particularly biogroup G1. *E. agglomerans* is chiefly characterized by the *absence of LDC, ODC and ADH* and by the synthesis of a nondiffusible yellow pigment. Biogroup 1 is usually yellow pigmented, has a strongly active gelatinase, lacks β-xylosidase, does not ferment D-sorbitol or mucate, and is inhibited by KCN. Pectinolytic, lipolytic and alginolytic activities have not been detected in *E. agglomerans*.

Most strains are sensitive to antibiotics, except for possible resistance to ampicillin and cephalothin and sometimes to carbenicillin and nitrofurañes.

Isolated from plants, flowers, seeds, vegetables, water, soil and foodstuffs. Some strains are of human and animal origin.

Table 5.22.
Main characteristics differentiating **Enterobacter cloacae, Enterobacter sakazakii** *and* **Enterobacter agglomerans**[a]

Characteristics	1. *E. cloacae*	2. *E. sakazakii*	3. *E. agglomerans*	
			Aerogenic Biogroups	Anaerogenic Biogroups
Gas from D-glucose	+	+	+	−
Yellow pigment	−	+	d	[+]
Decarboxylases:				
Lysine	−	−	−	−
Ornithine	+	+	[−]	−
Arginine	+	+	−	−
Acid from:				
Sorbitol	+	−	[+]	[−]
Mucate	d	−	d	d
Indole	−	d	d	d

[a] For symbols see Table 5.21.

Table 5.23.
Differentiation of the biogroups of anaerogenic strains of **Enterobacter agglomerans**[a]

Biogroup	Nitrate Reduction	Indole	Voges-Proskauer	No. of Strains Examined
1	+	−	+	157
2	+	−	−	52
3	−	−	−	21
4	−	+	+	19
5	+	+	−	19
6	−	−	+	12
7	+	+	+	8

[a] From Fife and Ewing (1972). Symbols: +, all strains positive in 24–48 h; −, all strains negative after 7 days.

Table 5.24.
Differentiation of the biogroups of aerogenic strains of **Enterobacter agglomerans**[a]

Biogroup	Indole	Voges-Proskauer	No. of Strains Examined
G1	−	+	33
G2	−	−	15
G3	+	−	15
G4	+	+	6

[a] From Fife and Ewing (1972). Symbols: +, all strains positive in 24–48 h; −, all strains negative after 7 days.

The mol% G + C of the DNA is 53–58 (Bd).
Type strain: ATCC 27155 (NCTC 9381, CDC 1461-67).

Further Comments

The synonymy of *E. agglomerans* with *Erwinia* species has already been discussed (see Taxonomic Comments). Also, a species called *Escherichia adecarboxylata* (see the chapter on the genus *Escherichia* in this *Manual*) probably belongs to the *Enterobacter agglomerans* complex (Bascomb et al., 1971).

4. **Enterobacter aerogenes** (Kruse 1896) Hormaeche and Edwards 1960, 72.[AL] (*Bacillus aerogenes* Kruse 1896, 340.)
a.e.ro'ge.nes. Gr. masc. n. *aer* air; Gr. v. *gennanio* to produce; M.L. adj. *aerogenes* gas-producing.
The characteristics are described in Table 5.20. *E. aerogenes* shares many biochemical characters with *Klebsiella pneumoniae*, such as acidification of many carbohydrates with gas, utilization malonate, and a positive LDC reaction. *Motility, ODC and urease* are the major characteristics to differentiate these two species (Table 5.25).
Most strains of *E. aerogenes* are resistant to ampicillin and cephalosporins and sensitive to carbenicillin.
Occur in water, sewage, soil, dairy products and the feces of man and animals.
The mol% G + C of the DNA is 53–54 (Bd).
Type strain: ATCC 13048 (NCTC 10006, CDC 819-56).

5. **Enterobacter gergoviae** Brenner, Richard, Steigerwalt, Asbury and Mandel 1980, 1.[VP]
ger.go'vi.ae. M.L. gen. n. *gergoviae* of Gergovie Highland; intended to pertain to the fact that the type strain was isolated from samples taken during a urinary infection outbreak in Clermont-Ferrand University Hospital near Gergovie Highland in France.
The characteristics are as described in Table 5.20. *E. gergoviae* is closest to *E. aerogenes* phenotypically (Richard et al., 1976) but is *urease-positive.* Table 5.20 indicates other characteristics that distinguish between these two species.
Most strains are susceptible to antibiotics, but strains isolated from a urinary infection outbreak in France were multiresistant.
Occur in various environmental sources such as cosmetics, water, etc. Have also been recovered from clinical specimens.
The mol% G + C of the DNA is 60 (Bd).
Type strain: CIP 76.01 (ATCC 33028; CDC 604-77).

Table 5.25.
Main Characteristics differentiating **Enterobacter aerogenes** *from* **Klebsiella pneumoniae**[a]

Characteristics	*E. aerogenes*	*K. pneumoniae*
Motility	+	−
Ornithine decarboxylase	+	−
Urease	−	+
Sorbose, acid	−	d
Lactose, acid	(+) or +	+
m-Hydroxybenzoate	+	−
Gelatinase	(+) or +	−
Carbenicillin	S	R
Cephalothin	R	S

[a] Symbols: +, all strains positive in 24–48 h; (+), delayed positive (positive between 3 and 7 days); −, all strains negative after 7 days; S, susceptible; R, resistant; d, differs among strains.

Other organisms belonging to the genus **Enterobacter**

Two newly described species of *Enterobacter* have been validly published and are distinct from each other and from other *Enterobacter* species on the basis of DNA relatedness. They can be differentiated phenotypically from other *Enterobacter* by their inability to grow at 41°C and by the characteristics listed in Table 5.20.

a. *Enterobacter intermedium* Izard, Gavani and Leclerc 1980, 601.[VP] (*Effective publication*: Izard, Gavini and Leclerc 1980, 51.)
in.ter.me'di.um. L. adj. *intermedium* intermediate.
Type strain: CIP 79-27 (CUETM 77-130; strain E86 of Gavini).

b. *Enterobacter amnigenus* Izard, Gavini, Trinel and Leclerc 1981, 37.[VP]
am.ni'ge.nus. L. adj. *amnigenus* coming from water.
The mol% G + C of the DNA is 60 (Bd).
Type strain: ATCC 33072 (CUETM 77-118).
Two other organisms presently listed under the genus *Erwinia* belong to the genus *Enterobacter* on the basis of DNA relatedness.

c. *Erwinia dissolvens* (Rosen 1922) Burkholder 1948, 472.[AL] (*Pseudomonas dissolvens* Rosen 1922, 497.)
dis.sol'vens. L. part. adj. *dissolvens* dissolving.
This organism belongs to the genus *Enterobacter* (Waldee, 1945; Dye, 1969; Steigerwalt et al., 1976) as a new species or as a biogroup of *E. cloacae.* Its DNA is 60–80% related to DNA from *E. cloacae* and the two organisms are very similar biochemically (Steigerwalt et al., 1976).
Type strain: ATCC 23373.

d. *Erwinia nimipressuralis* Carter 1945, 423.[AL]
ni.mi.pres.su.ra'lis. L. adv. *nimis* overmuch; L. n. *pressura* pressure; M.L. adj. *nimipressuralis* with excessive pressure.
This organism belongs to the genus *Enterobacter* (Graham, 1964; Dye, 1969; Steigerwalt et al., 1976) as a new species or as a biogroup of *E. cloacae.* Its DNA is 55–65% related to that of *E. cloacae* and *E. dissolvens* (Steigerwalt et al., 1976). *E. nimipressuralis* is negative in sucrose and raffinose reactions—characteristics which separate it from *E. cloacae* (Steigerwalt et al., 1976). It was reported as the causative agent of "wetwood" disease in elm trees (Carter, 1945).
Type strain: ATCC 9912.

Genus VII. **Erwinia** *Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209*[AL]

R. A. LELLIOTT AND ROBERT S. DICKEY

(Includes *Pectobacterium* Waldee 1945, 469.[AL])

Er.wi'ni.a. M.L. fem. n. *Erwinia*; named after Erwin F. Smith.

Straight rods, 0.5–1.0 × 1.0–3.0 μm; occur singly, in pairs and sometimes in short chains. Gram-negative. **Motile** (one exception) by peritrichous flagella. **Facultatively anaerobic**, but anaerobic growth by some species is weak. Optimum temperature, 27–30°C; maximum varies between 32°C and at least 40°C. **Oxidase-negative. Catalase-positive. Acid is produced from** fructose, galactose, D-glucose, β-metylglucoside and sucrose. **Utilize** acetate, fumarate, gluconate, malate and succinate, but not benzoate, oxalate, or propionate as carbon- and energy-yielding sources. **Associated with plants as pathogens, saprophytes, or as constituents of the epiphytic flora.** At least one species has also been isolated from human and animal hosts. The mol% G + C of the DNA is 50–58 (T_m, Bd).

Type species: *Erwinia amylovora* (Burrill 1882) Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209.

Further Descriptive Information

Acid is usually produced from mannitol, mannose, ribose and sorbitol, but rarely from adonitol, dextrin, dulcitol or melezitose. Gas production is comparatively weak or absent. Decarboxylases for arginine, lysine or ornithine cannot be detected by Møller's method (Møller, 1955) except in a few (usually 5% or less) strains of *E. carotovora* and *E. chrysanthemi*. Formation of putrescine occurs when the amino acids are decarboxylated under aerobic conditions (Zherebilo and Gvozdyak, 1976). Glutamic acid is not decarboxylated. Urease and lipases rarely are produced. Additional characters of the species and subspecies of the genus are given in Tables 5.26 to 5.28 with data for *E. cypripedii*, *E. nigrifluens*, *E. quercina*, *E. rubrifaciens*, *E. salicis*, *E. tracheiphila* and *E. uredovora* based on relatively small numbers of strains for each species.

Pectate lyases are produced by strains of *E. carotovora* (Mount et al., 1970), *E. carotovora* subsp. *atroseptica* (Hall and Wood, 1970), *E. chrysanthemi* (Garibaldi and Bateman, 1971), and *E. rubrifaciens* (Gardner and Kado, 1976). Cellulase (Cx) also is produced by strains of *E. carotovora*, *E. carotovora* subsp. *atroseptica*, and *E. chrysanthemi* in the presence of carboxymethyl cellulose (El-Helaly et al., 1979).

Fermentation end products from glucose are CO_2 and different combinations of succinate, lactate, formate and acetate; some form 2,3-butanediol and some ethanol (White and Starr, 1971). Starch is not hydrolyzed beyond dextrins.

Naturally occurring plasmids have been detected in strains of *E. amylovora*, *E. carotovora*, *E. chrysanthemi* and *E. herbicola*, and plasmids from bacteria other than *Erwinia* have been introduced into strains of the foregoing *Erwinia* species and strains of *E. carotovora* subsp. *atroseptica*, *E. nigrifluens*, and *E. uredovora* (Lacy and Leary, 1979; Chatterjee and Starr, 1980). Plasmid-mediated transfer of chromosome genes by conjugation also has been reported for strains of *E. amylovora*, *E. carotovora*, *E. chrysanthemi* and *E. herbicola*.

Virulent or temperate phages have been isolated, characterized and reported to be active against strains of *E. amylovora* (Ritchie and Klos, 1979), *E. carotovora* (Chapman et al., 1951; Faltus and Kishko, 1980), *E. chrysanthemi* (Paulin and Nassan, 1978), *E. herbicola* (Harrison and Gibbins, 1975), *E. nigrifluens* and *E. rubrifaciens* (Zeitoun and Wilson, 1969). Bacteriocinogeny or production of bacteriocin-like substances has been noted for strains of *E. carotovora* (Itoh et al., 1978), *E. chrysanthemi* (Echandi and Moyer, 1979), *E. herbicola* (Beer and Vidaver, 1978), and *Erwinia* species from sugar beet (Stanghellini et al., 1977).

Antisera prepared against live or heat-killed cells, nonpurified or purified immunogens have been used for the differentiation or identification of all *Erwinia* species except *E. ananas*, *E. cypripedii*, *E. mallotivora*, *E. rhapontici* and *E. uredovora* (Elrod, 1946; DeKam, 1976; Schaad, 1979). Serogroups have been determined for *E. carotovora* (De Boer et al., 1979) and *E. chrysanthemi* (Samsun and Nassan-Agha, 1978; Yakrus and Schaad, 1979).

Erwinia species cause plant diseases which include blights, cankers, die back, leaf spots, wilts, discoloration of plant tissues, and soft rots variously described as stalk rot, crown rot, stem rot, or fruit collapse. Ingress by the pathogen generally occurs through natural openings and wounds. *Erwinia uredovora* is a parasite of rust fungi and multiplies in the plant tissue infected by the rust organism (Hevesi and Mashaal, 1975). *Erwinia tracheiphila* overwinters in the bodies of cucumber beetles (*Diabrotica vittata* Fabr. and *D. duodecimpunctata* Oliv.) (Leach, 1964), whereas *E. stewartii* overwinters primarily in a flea beetle (*Chaetocnema pulicaria* Melsh.) (Pepper, 1967). Strains of *E. herbicola* are common in the epiphytic microflora of plants; instances have been reported in which *E. herbicola* has produced symptoms on plants, sometimes possibly in association with other phytopathogenic bacteria (Gibbins, 1978).

Enrichment and Isolation Procedures

The pathogens generally can be easily isolated. The affected plant material should be washed in tap water, followed by sterile water, and dried with paper toweling. Surface sterilization (3 min in 1:10 dilution of 5.25% active sodium hypochlorite) sometimes is detrimental for isolation. Affected tissue is removed from a young lesion or the edge of older necrotic areas by a sterile scalpel; the tissue is comminuted in sterile water, saline, or buffer solution and is streaked onto a solid medium, such as nutrient agar or YDC (Dye, 1968). The isolation of *E. tracheiphila* is more easily accomplished by aseptically cutting the affected stem, placing the two cut stem surfaces together, and gently pulling apart, removing a portion of the threads of bacteria and placing the bacteria in nutrient broth or onto a solid medium (Burkholder, 1960). The delicate growth of *E. tracheiphila* will appear in 3 or 4 days; frequent transfer is necessary, but virulence may be reduced or lost with repeated transfers.

The isolation of some *Erwinia* species can be facilitated by use of selective-differential media, but such media are usually not necessary. *Erwinia amylovora*, *E. herbicola*, *E. nigrifluens*, *E. quercina* and *E. rubrifaciens* will grow on MS medium (Miller and Schroth, 1972) and produce characteristic colonies. Sorbitol is substituted for mannitol in the MS medium for the isolation of *E. amylovora* (Schroth and Hildebrand, 1980). The medium of Crosse and Goodman (Crosse and Goodman, 1973) also can be used for *E. amylovora*. Selective media have been developed for the isolation of pectolytic erwinias (Kelman and Dickey, 1980). The CVP medium containing crystal violet and sodium polypectate (Cuppels and Kelman, 1974) is commonly used. Although pectolytic pseudomonads also will grow on CVP, they can be eliminated by adding manganese. A soluble pink pigment is produced by *E. rubrifaciens* and *E. rhapontici* grown on YDC.

Maintenance Procedures

Stock cultures of *Erwinia* species should be grown on standard media of choice at 25–30°C until good growth occurs. The cultures can be maintained for short term storage in a refrigerator (4–5°C); some strains of *E. chrysanthemi* are nonviable after 3 or 4 weeks at 4°C, but remain viable for longer periods when stored at 12°C.

For long term preservation, erwinias can be successfully stored as lyophilized cultures usually suspended in equal amounts of 10% glucose and 10% peptone (Ferguson and Nuttall, 1964; Lelliott, 1965). Strains also have been stored in distilled water at 10°C by the method of DeVay and Schnathorst (1963), in soil or under mineral oil (Lelliott, 1965), in liquid nitrogen (cells suspended in 10% skim milk) (Moore and Carlson, 1975), and in glycerol at −70°C or on silica gel at −20°C (Sleesman and Leben, 1978).

Taxonomic Comments

The taxonomy of the genus *Erwinia* and designation of species in the genus has been complicated by the heterogeneity of the strains included in the taxon. It has been suggested that members of the genus be placed into new groupings with other members of the *Enterobacteriaceae* (Starr and Mandel, 1969; White and Starr, 1971). This concept also is supported by studies of selected strains by DNA/DNA homology (Gardner and Kado, 1972), DNA relatedness (Brenner, Fanning and Steigerwalt, 1974) and DNA/DNA segmental homology (Murata and Starr, 1974). The data for the successful implementation of this proposal currently are however, not available. Therefore, the order of the species and subspecies used herein reflects relatedness to the type species, *E. amylovora*, based on cluster analysis using 54 phenotypic characteristics (Dickey, unpublished observations). The results of four numerical analyses have shown that a different relationship between the various nomenspecies was indicated by each method of analysis (Dye, 1981).

The heterogeneity within the genus also is reflected in the genetic clusters which have been proposed. Waldee (1945) suggested that *Erwinia* should be limited to pathogens (*E. amylovora*, *E. salicis* and

E. tracheiphila) that cause necrotic or wilt diseases, utilize a restricted range of carbon compounds and usually require organic nitrogen compounds for growth; and that the biochemically more active soft rotting pathogens (*E. carotovora* and *E. chrysanthemi*) should be placed in a separate genus *Pectobacterium*. Although some workers have supported this suggestion (Brenner et al., 1973, 1974), it has not been generally accepted because there are species taxonomically intermediate between these two groups, and there are pathogens that resemble *E. carotovora* in most of their characteristics but do not cause rots. A proposal also has been made whereby one genus is retained and the organisms are separated into three groups, namely, the Amylovora, Herbicola, and Carotovora groups (Dye, 1968, 1969); however, these groupings are subject to the same inconsistencies mentioned above. A core of relatedness and genetic clusters have been demonstrated for strains and nomenspecies of *Erwinia* by molecular hybridization and segmental homology, although the affinities between most members of the genus are no greater than for other enterobacteria (Gardner and Kado, 1972; Brenner et al., 1973; Brenner et al., 1974; Murata and Starr, 1974; Azad and Kado, 1980).

Erwinia herbicola includes an assortment of yellow and nonpigmented strains from plant lesions, plant surfaces, man and animals, and occasionally from soil, water and air. These organisms previously have been assigned to various genera and species (Dye, 1969; Gibbins, 1978). Strains of interest to phytopathologists have been placed in the genus *Erwinia*. Ewing and Fife (1972) proposed that strains from clinical sources be designated as *Enterobacter agglomerans* because the characteristics of the organisms were in conformity with the genus *Enterobacter* (see the article on *Enterobacter* in this *Manual*). It currently is difficult, if not impossible, to distinguish strains from different sources due to the diversity in this group of organisms. Further phenotypic and genotypic studies must be done to define the groups now referred to as *E. agglomerans* and *Erwinia* species. See the family *Enterobacteriaceae* for additional discussion of this problem.

Strains of *E. chrysanthemi* have been isolated from numerous plant species and cultivars (Dickey, 1981). Six pathovars (pv. *chrysanthemi*, pv. *dianthicola*, pv. *dieffenbachiae*, pv. *paradisiaca*, pv. *parthenii* and pv. *zeae*) have been designated for *E. chrysanthemi* (Dye et al., 1980). The relationship between pathogenicity, phenotypic properties and serological reactions of strains of the pathovars is not entirely clear (Samson and Nassan-Agha, 1978; Yakrus and Schaad, 1979; Dickey, 1981).

Differentiation and characteristics of the species of the genus Erwinia

The differential characteristics of the species of *Erwinia* are given in Tables 5.26 to 5.28. Only small numbers of strains of *E. tracheiphila*, *E. rubrifaciens*, *E. quercina*, *E. salicis*, *E. cypripedii*, *E. nigrifluens* and *E. uredovora* have been studied, and data for these species should be treated with reserve.

List of the species and subspecies of the genus Erwinia

1. **Erwinia amylovora** (Burrill 1882) Winslow, Broadhurst, Buchanan, Krumwiede, Rogers and Smith 1920, 209[AL] (*Micrococcus amylovorus* Burrill 1882, 134.)

a.my.lo'.vo.ra. Gr. n. *amylum* starch; L. v. *voro* to devour; M.L. fem. adj. *amylovora* starch-destroying.

The characteristics are as given for the genus and as listed in Tables 5.26–5.28.

Colonies on 5% sucrose nutrient agar are typically white, domed, shining, mucoid (levan type) with radial striations and a dense flocculent center or central ring after 2 or 3 days at 27°C. Non-levan forms are isolated rarely. (See Enrichment and Isolation Procedures for selective media.)

Agglutination with *E. amylovora* antiserum is the most rapid and accurate method of determination (Lelliott, 1968); the species is serologically homogeneous and has few agglutinogens in common with related species or with the saprophytes found in diseased material.

Causes a necrotic disease (fireblight) of most species of the *Pomoideae* and of some species in other subfamilies of the *Rosaceae*. A *forma specialis* has been described from raspberry (*Rubus idaeus*) by Starr and Folsom (1951).

The mol% G + C of the DNA of seven strains ranges from 53.6–54.1 (Bd).

Type strain: ATCC 15580 (strain BS1114 of Martinec and Kocur, 1964).

2. **Erwinia tracheiphila** (Smith 1895) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 173.[AL] (*Bacillus tracheiphilus* Smith 1895, 364.)

tra.che.i'phi.la. L. n. *trachia* the windpipe; Gr. adj. *philus* loving; M.L. adj. *tracheiphila* trachea-loving, i.e. growing in the tracheiphila of the vascular bundles.

The characteristics are as given for the genus and as listed in Tables 5.26–5.28.

Grows very poorly on nutrient agar but moderately well on yeast extract glucose chalk agar (YDC) or glucose nutrient agar.

Causes a vascular wilt of *Cucurbita* species.

The mol% G + C of the DNA of three strains ranges from 50–52 (Bd).

Type strain: NCPPB 2452 (Approved Lists, 1980).

3. **Erwinia mallotivora** Goto 1976, 472.[AL]

mal.lo.ti'vo.ra. M.L. n. *Mallotus* a genus of trees; L. v. *voro* to devour; M.L. adj. *mallotivora Mallotus*-destroying.

The characteristics are as described for the genus and as listed in Tables 5.26–5.28.

Colonies on nutrient agar without sucrose are white, raised, transparent, and circular with smooth surfaces and entire margins after 2 days. Colonies on nutrient agar with 5% sucrose are flat, white, circular with entire margins and smooth surfaces, butyrous, and transparent after 1 day; after 4 days colonies are domed, circular, white, mucoid, and translucent, and sometimes possess radial striations.

Causes a leaf spot of Akamegashiwa (*Mallotus japonicus*).

The mol% G + C of the DNA of two strains is 49.8 and 51.0 (Bd).

Type strain: ATCC 29573 (strain AM1 of Goto, 1976).

4. **Erwinia rubrifaciens** Wilson, Zeitoun and Fredrickson 1967, 621.[AL] (*Erwinia amylovora* var. *rubrifaciens* (Dye 1968, 605.)

rub.ri.fac'i.ens. L. adj. *ruber* red; L. v. *facio* make; M.L. part. adj. *rubrifaciens* red-producing.

The characteristics are as given for the genus and as listed in Tables 5.26–5.28.

Grows poorly on nutrient agar, but well on yeast exract glucose chalk agar (YDC) on which colonies are cream to yellow, low convex, smooth, shining with entire margins. Craters form around colonies on the polypectate gel B and C of Hildebrand (1971).

Causes a phloem necrosis of Persian walnut trees (*Juglans regia*).

The mol% G + C of the DNA of three strains ranges from 52.0–52.6 (Bd).

Type strain: ATCC 29291 (Dye, 1968).

5. **Erwinia quercina** Hildebrand and Schroth 1967, 253.[AL] (*Erwinia amylovora* var. *quercina* (Hildebrand and Schroth 1967) Dye 1968, 605.)

Table 5.26.
Cultural, physiological and biochemical characteristics of the species of the genus **Erwinia**[a,b]

Characteristics	1. *E. amylovora*	2. *E. tracheiphila*	3. *E. mallotivora*	4. *E. rubrifaciens*	5. *E. quercina*	6. *E. salicis*	7. *E. herbicola*	8. *E. ananas*	9. *E. rhapontici*	10. *E. carotovora*	11. *E. chrysanthemi*	12. *E. cypripedii*	13. *E. nigrifluens*	14. *E. stewartii*	15. *E. uredovora*
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
Anaerobic growth	W	W	+	+	+	W	+	+	+	+	+	+	+	+	+
Growth factors required[c]	+	+	+	−	+	−	−	−	−	−	−	−	−	−	−
Pink diffusible pigment[d]	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−
Blue pigment[e]	−	−	−	−	−	−	−	−	−	−	d	−	−	−	−
Yellow pigment[f]	−	−	−	−	−	−	+	+	−	−	−	−	−	+	+
Mucoid growth[g]	+	−	+	+	+	+	d	+	+	d	d	d	−	+	−
Symplasmata[h]							d	−						−	d
Growth at 36°C	−	−	−	+	+	−	+	+	d	d	+	+	+	d	+
H_2S from cysteine[i]	−	+	−	+	+	+	+	d	+	+	+	+	+	−	−
Reducing substances from sucrose[j]	+	d	+	−	+	+	d	+	d	d	−	−	−	d	+
Acetoin[i]	+	d	+	−	+	+	+	+	+	+	+	−	+	−	+
Urease[i]	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−
Pectate degradation[k]	−	−	−	+	−	+	−	−	−	+	+	−	−	−	−
Gluconate oxidation[l]	−	−	−	−	−	−	−	−	d	−	−	+	−	−	−
Gas from D-glucose[m]	−	−	−	−	−	−	−	−	−	d	+	+	−	−	−
Casein hydrolysis[i]	−	−	−	−	−	−	−	−	−	d	d	−	−	−	−
Growth in KCN broth	−	−	−	−	−	−	−	−	+	d	d	+	−	−	−
Cotton seed oil hydrolysis[i]	−	−	−	−	−	−	−	−	d	d	d	+	−	−	−
Gelatin liquefaction[i]	+	−	−	−	−	−	+	+	−	+	+	−	−	−	+
Phenylalanine deaminase[n]	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−
Indole test[o]	−	−	−	−	−	−	−	+	−	−	+	−	−	−	+
Nitrate reduction[i]	−	−	−	−	−	−	+	−	+	+	+	+	−	−	+
Growth in 5% NaCl		−	−				+	+	+	+	d	+		+	+
Deoxyribonuclease (DNase)[p]	−	−		−	−	−	−	−	−	−	−	−	−	−	+
Phosphatase[q]			−						d	−	+	d			
Lecithinase[r]									−	−	+	−			
Sensitivity to erythromycin(15 μg/disk)									+	−	+	+			

[a] Data mostly from Dye (1968, 1969) with supplemental data from Graham (1972), Goto (1976), Sellwood and Lelliott (1978), Dickey (1979) and Dickey and Victoria (1980). For invariant characters see generic description.
[b] Symbols: +, 80% or more of strains positive; −, 20% or less of strains positive; d, 21–79% of strains positive; W, weak growth; blank space, insufficient or no data.
[c] *E. amylovora* requires nicotinic acid. Other growth factor-requiring species will grow in an inorganic salts medium with utilizable C source and yeast extract; their exact requirements are not known.
[d] On 1% yeast extract, 1% D-glucose, 2% ppt. chalk, 2% agar (YDC). Pigment production by *E. rhapontici* is more consistent on media containing 2% sucrose, 0.5% peptone, 0.05% K_2HPO_4, 0.025 $MgSO_4$, 2% agar (pH 7.2–7.4), or 5% sucrose nutrient agar.
[e] On YDC (see footnote *d* above) after 5–10 days at 27°C.
[f] On nutrient agar. Nonpigmented strains of *E. herbicola* occur (Billing and Baker, 1963) but their frequency in relation to pigmented strains is not known.
[g] On 5% sucrose nutrient agar.
[h] See Graham and Hodgkiss (1967).
[i] By the methods of Dye (1968).
[j] After 2 days shake culture at 27°C in 4% sucrose, 1% peptone, 0.5 beef extract broth. The production of an orange or brown color (with or without precipitate) with an equal volume of Benedict's quantitative reagent after 10 min in a boiling water bath constitutes a positive reaction.
[k] In 3 days at 27°C on Paton's medium (Paton, 1959).
[l] After 4 days shake culture at 27°C in the medium of Shaw and Clarke (1955) and tested and read as footnote *j* above.
[m] In the sealed tube of Hugh and Leifson's (1953) O/F medium. *E. quercina* and *E. rubrifaciens* produce small amounts of gas (possibly from peptone) on some other media.
[n] On phenylalanine agar test No. 18, 2–3 days at 27°C (Report, 1958). The reaction is weaker than that given by *Proteus* species.
[o] After 2 and 5 days at 27°C in 1% tryptone, 0.1% tryptophan broth and tested with Kovacs' reagent. *E. chrysanthemi* probably converts tryptophan to α-methyl indole and not indole (Lelliott, 1956).
[p] On DNase test agar after 2 days at 27°C (Graham and Hodgkiss, 1967).
[q] As described by Cowan and Steel (1965), using 0.05% sodium phenolphthalein diphosphate agar after 2 days at 27°C.
[r] On egg-yolk agar after 7 days at 27°C.

Table 5.27.
Acid production from organic compounds by **Erwinia** *species*[a,b]

Compound	1. *E. amylovora*	2. *E. tracheiphila*	3. *E. mallotivora*	4. *E. rubrifaciens*	5. *E. quercina*	6. *E. salicis*	7. *E. herbicola*	8. *E. ananas*	9. *E. rhapontici*	10. *E. carotovora*	11. *E. chrysanthemi*	12. *E. cypripedii*	13. *E. nigrifluens*	14. *E. stewartii*	15. *E. uredovora*
Melibiose	−	−	−	−	−	+	−	+	+	+	+	+	+	+	+
Inositol	−	−	−	−	−	+	−	+	+	d	d	+	+	−	+
Raffinose	−	−	−	−	−	+	d	+	+	+	+	−	+	+	+
Inulin	−	−	−	−	−	−	+	d	+	−	d	−	−	d	+
Starch	−	−	−	−	−	−	+	+	+	−	−	−	−	−	+
Maltose	−	−	−	−	−	−	+	+	+	d	−	+	−	−	+
L-Arabinose	d	−	−	+	−	−	+	+	+	+	+	+	+	+	+
Sorbitol	d	−	−	+	+	+	+	+	+	+	+	+	+	+	+
Ribose	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannose	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	−	−	(+)	−	−	−	−	+	+	+	+	+	−	−	+
Lactose	−	−	−	−	−	−	d	+	+	+	d	−	−	+	+
Rhamnose	−	−	−	−	−	−	+	d	+	+	+	+	+	−	+
Esculin	−	−	−	−	+	+	d	d	+	+	+	+	+	−	d
Salicin	−	−	−	−	+	+	d	+	+	+	+	+	+	−	d
Xylose	−	−	+	−	−	−	+	+	d	+	+	+	+	+	+
Trehalose	+	−	+	−	−	−	+	+	+	+	−	+	+	+	+
Dulcitol	−	−	−	−	−	−	+	−	d	−	−	−	−	−	−
Glycerol	−	−	(+)	d	+	d	−	+	+	d	+	d	+	−	+
Adonitol	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Dextrin	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+
Melezitose	−	−	−	−	−	−	−	−	d	−	−	−	−	−	+
α-methyl glucoside	−	−	−	+	+	−	−	−	d	d	−	−	−	−	−

[a] After 7-days growth at 27°C in unshaken aqueous solution of 1% organic compound, 1% peptone with bromcresol purple as an indicator. *E. tracheiphila* grows very slowly in the medium.
[b] Symbols: +, 80% or more of strains positive; −, 20% or less of strains positive; (+), delayed positive reaction; d, 21–79% of strains positive. For invariant characters, see generic description. Data mostly from Dye (1968, 1969) with supplemental data from Graham (1972), Goto (1976), Sellwood and Lelliott (1978), Dickey (1979) and Dickey and Victoria (1980).

quer.ci'na. L. n. *quercus* oak; L. suff. *-ina* belonging to; M.L. part. adj. *quercina* oak-belonging.

The characteristics are as given for the genus and as listed in Tables 5.26–5.28.

Growth on potato glucose peptone calcium carbonate (PGPC) agar is luxuriant and after 24 h colonies are white, circular and raised with entire margins. Craters form around colonies on polypectate gel of Hildebrand (1971).

Small amounts of gas are produced (possibly from peptone) in a glucose peptone medium and in PGPC.

Superficially rots onion (but not potato) slices and induces profuse lateral root development in 3 or 4 days on slices of carrot, turnip or beet.

Causes copious oozing of sap from acorns and, by artificial inoculation, shoot blight of *Quercus agrifolia* and *Q. wislizeni*.

The mol% G + C of the DNA of two strains is 54.6 and 55.1 (Bd).

Type strain: ATCC 29281 (Dye, 1968).

6 **Erwinia salicis** (Day 1924) Chester 1939, 406.[AL] (*Bacterium salicis* Day 1924, 14.)

sa'li.cis. L. n. *salix* the willow; L. gen. n. *salicis* of the willow.

The characteristics are as given for the genus and as listed in Tables 5.26–5.28.

Grows poorly on nutrient agar but moderately well on yeast extract glucose chalk agar (YDC) or on glucose nutrient agar.

Colonies on 0.5% starch potato agar (pH 6.5) are yellowish in 2–3 days. A bright yellow pigment is produced on autoclaved potato tissue. Craters form around colonies on the pectate gel of Paton (1959).

Causes a vascular wilt of *Salix* species.

The mol% G + C of the DNA of two strains is 51.3 and 51.5 (Bd).

Type strain: ATCC 15712 (Martinec and Kocur, 1963).

7. **Erwinia herbicola** (Löhnis 1911) Dye 1964, 268.[AL] (*Bacterium herbicola* Löhnis 1911, 141; *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972, 10.)

her.bi'co.la. L. n. *herba* grass, green plants; L. suff. *-cola* dweller; M.L. n. *herbicola* grass-dweller.

The characteristics are as given for the genus and as listed in Tables 5.26–5.28.

The yellow (YC) and nonpigmented (DC) *Erwinia*-like organisms from plant sources described by Billing and Baker (1963) are included in this species.

Colonies of most strains are yellow; nonpigmented forms have been isolated and may be common. Small craters form around colonies on polypectate gel C of Hildebrand (1971).

Exists on plant surfaces and as secondary organisms in lesions caused by many plant pathogens. Some strains (syn. *Erwinia milletiae*) are reported to cause galls on *Milletia japonica*, some on *Wistaria floribunda* and *W. brachybotrys* (Goto et al., 1980) and some (syn. *Agrobacterium gypsophilae*) to cause galls on *Gypsophila paniculata*. Has been isolated from water (syn. *Flavobacterium rhenanum*), the enteric tract of man (syn. *Bacterium typhi flavum*, *Enterobacter* pigmentées anaérogènes Le Clerc, 1962, and see Gilardi et al., 1970), from septic tonsils of man, from the spleen and liver of symptomless deer (Muraschi et al., 1965)

Table 5.28.
Utilization of some organic compounds as a source of carbon and energy for **Erwinia** *species*[a,b]

Species	Citrate	Formate	Lactate	Tartrate	Galacturonate	Malonate
1. *E. amylovora*	+	+	+	−	−	−
2. *E. tracheiphila*	d	d	−	−	−	−
3. *E. mallotivora*	+	−	−	−	−	−
4. *E. rubrifaciens*	+	+	+	+	−	−
5. *E. quercinia*	+	+	+	−	−	−
6. *E. salicis*	−	−	−	−	−	−
7. *E. herbicola*	+	+	+	d	−	d
8. *E. ananas*	+	+	+	+	d	−
9. *E. rhapontici*	+	+	+	d	d	+
10. *E. carotovora*	+	+	+	−	d	−
11. *E. chrysanthemi*	+	+	+	d	d	+
12. *E. cypripedii*	+	+	+	+	+	d
13. *E. nigrifluens*	−	+	+	+	−	−
14. *E. stewartii*	+	+	+	+	−	−
15. *E. uredovora*	+	+	+	+	−	−

[a] In 21 days at 27°C on OY medium (Dye, 1968).
[b] Symbols: +, 80% or more of strains positive; −, 20% or less of strains positive; d, 21–79% of strains positive. Data mostly from Dye (1968, 1969) with supplemental data from Graham (1972), Goto (1976), Sellwood and Lelliott (1978), Dickey (1979) and Dickey and Victoria (1980). For invariant characteristics see generic description.

Table 5.29.
Characteristics differentiating the subspecies of **Erwinia carotovora**[a]

Characteristics	10a. *E. carotovora* subsp. *carotovora*	10b. *E. carotovora* subsp. *atroseptica*
Mucoid growth	d	−
Growth at 36°C	+	−
Reducing substances from sucrose	−	+
Casein hydrolysis	+	d
Cotton seed oil hydrolysis	d	−
Acid production from:		
Inositol	d	−
Maltose	−	+
Glycerol	+	d
α-Methyl glucoside	−	+
Utilization of galacturonate as a carbon and energy source	+	d

[a] For symbols and conditions see footnotes to Tables 5.26 to 5.28.

and from man and animals in the role of opportunistic pathogens (Gibbins, 1978; von Graevenitz, 1977).

The mol% G + C of the DNA of 30 strains ranges from 52.6–57.7 (Bd).

Type strain: NCPPB 2971 (Approved Lists, 1980).

8. **Erwinia ananas** Serrano 1928, 271.[AL]

a'na.nas. M.L. n. *Ananas* generic name of the pineapple.

The characteristics are as described for the genus and as listed in Tables 5.26–5.28.

The original description by Serrano (1928) is indistinguishable from *E. herbicola*, but studies of recent isolates of this pineapple pathogen indicate that it should be regarded as a distinct species.

Causes rot of pineapple (*Ananas sativus*) fruitlets.

The mol% G + C of the DNA of four strains is 53.1–54.1 (Bd).

Type strain: NCPPB 1846 (Approved Lists, 1980).

9. **Erwinia rhapontici** (Millard 1924) Burkholder 1948, 475.[AL] (*Phytomonas rhapontica* (*sic*) Millard 1924, 11; *Pectobacterium rhapontici* (Millard 1924) Patel and Kulkarni 1951, 80[AL]; *Erwinia carotovora* var. *rhapontici* (Millard 1924) Dye 1969, 93.)

rha.pon'ti.ci. M.L. n. *rhaponticum* specific epithet of *Rheum rhaponticum*, rhubarb; M.L. gen. n. *rhapontici* of rhubarb.

The characteristics are as described for the genus and as listed in Tables 5.26–5.28.

Rots potato, onion and cucumber slices slowly, weakly and erractically (Sellwood and Lelliott, 1978).

Causes a crown rot of rhubarb (*Rheum rhaponticum*), pink grain of wheat (Roberts, 1974), internal browning of hyacinth and occurs epiphytically and saprophytically in lesions caused by other bacteria (Sellwood and Lelliott, 1978).

The mol% G + C of the DNA of three strains ranges from 51.0–53.1 (Bd).

Type strain: ATCC 29283 (Approved Lists, 1980).

10. **Erwinia carotovora** (Jones 1901) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 171.[AL] (*Bacillus carotovorus* Jones 1901, 12; *Pectobacterium carotovorum* (Jones 1901) Waldee 1945, 469[AL]; *E. carotovora* var. *carotovora* Dye 1969, 81.)

ca.ro.to'vo.ra. L. n. *carota* carrot; L. v. *voro* to devour; M.L. adj. *carotovora* carrot-devouring.

The characteristics are as described for the genus and as listed in Tables 5.26–5.28.

Causes rotting, particularly of storage tissues, of a wide variety of plants and causes a vascular and parenchymatal disease (blackleg) of potato plants.

The species is divided into two subspecies.

The mol% G + C of the DNA ranges from 50.5–53.1 (Bd).

Type strain: ATCC 15713 (Martinec and Kocur, 1963).

10a. **Erwinia carotovora** subspecies **carotovora** (Jones 1901) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 171.[AL]

Characteristics distinguishing this subspecies from the subspecies *atroseptica* are indicated in Table 5.29. Gas production from carbohydrates is erratic; some strains (syn. *E. aroideae*) are anaerogenic when isolated, others produce moderate or small amounts of gas and often become anaerogenic after prolonged culture.

Causes rotting, particularly of storage tissues, of a wide variety of plants.

The mol% G + C of the DNA of 11 strains ranges from 50.5–53.1 (Bd).

Type strain: ATCC 15713.

10b. **Erwinia carotovora** subspecies **atroseptica** (van Hall 1902) Dye 1969, 81.[AL] (*Bacillus atrosepticus* van Hall 1902, 134.)

at.ro.sep'ti.ca. L. adj. *ater* black; Gr. adj. *septicus* producing a putrefaction; M.L. adj. *atrospetica* producing a black rot.

Characteristics distinguishing this subspecies from the subspecies *carotovora* are indicated in Table 5.29.

Causes a vascular and parenchymatal disease (blackleg) of potato (*Solanum tuberosum*) plants and a storage rot of potato tubers.

The mol% G + C of the DNA of two strains is 51.3 and 53.1 (Bd).

Type strain: NCPPB 549 (Approved Lists, 1980).

Further Comments

A third subspecies of *E. carotovora* has recently been described: "*Erwinia carotovora* subspecies *betavasculorum*" Thomson, Hildebrand and Schroth 1981, 1040.

Characteristics distinguishing this subspecies from subspecies *carotovora* and *atroseptica* are growth at 36°C, reducing substances formed from sucrose, utilization of inositol, maltose, glycerol, and α-methyl glucoside, but not galacturonate. Other nutritional and physiological

characteristics useful for distinguishing the subspecies include: utilization of D-lactate, ethanol, L-lysine, palatinose and D-asparagine, but not cellobiose, melibiose, malonate and raffinose; no production of indole, phosphatase or gas from glucose; and resistance to erythromycin.

Causes soft rot of sugar beet.

The mol% G + C of the DNA of three strains is 54.4–54.7 (T_m).

Designated type strain: NCPPB 2795 (Thomson et al., 1981).

11. **Erwinia chrysanthemi** Burkholder, McFadden and Dimock 1953, 526.[AL] [*Pectobacterium chrysanthemi* (Burkholder, McFadden and Dimock 1953) Brenner, Steigerwalt, Miklos and Fanning 1973, 205.[AL]) (Subj. syns.: *Erwinia carotovora* var. *paradisiaca* Victoria and Barros 1969, 189; *Erwinia paradisiaca* Fernández-Borrero and López-Duque 1970, 22.)

chrys.an'.the.mi. M.L. n. *Chrysanthemum* generic name; M.L. gen. n. *chrysanthemi* of chrysanthemums.

The characteristics are as described for the genus and as listed in Tables 5.26–5.28.

Colonies on potato-glucose-agar (pH 6.5) are characteristically umbonate with undulate to coralloid margins ("fried egg") at 3–6 days of growth.

Causes vascular wilts or parenchymatal necroses of a wide range of plant species and cultivars (Dickey, 1981). There is evidence for differentiation into pathovars (see Taxonomic Comments).

The mol% G + C of the DNA of six strains is 55.1–57.1 (Bd).

Type strain: ATCC 11663 (Approved Lists, 1980).

12. **Erwinia cypripedii** (Hori 1911) Bergy, Harrison, Breed, Hammer and Huntoon 1923, 171.[AL] (*Bacillus cypripedii* Hori 1911, 91; *Erwinia carotovora* var. *cypripedii* (Hori 1911) Dye 1969, 93: *Pectobacterium cypripedii* (Hori 1911) Brenner, Steigerwalt, Miklos and Fanning 1973, 205.[AL])

cyp.ri.ped'i.i. M.L. n. *Cypripedium* generic name; M.L. gen. n. *cypripedii* of cypripedium orchids.

The characteristics are as described for the genus and as listed in Tables 5.26–5.28.

Causes a brown rot of cypripedium orchids (*Cypripedium* spp.).

The mol% G + C of the DNA of two strains is 54.1 and 54.6 (Bd).

Type strain: PDDCC 1591 (Approved Lists, 1980).

13. **Erwinia nigrifluens** Wilson, Starr and Berger 1957, 673.[AL] (*Erwinia amylovora* var. *nigrifluens* Dye 1968, 605.)

ni.gri.flu'ens. L. adj. *niger, nigra* black; L. v. *fluo* flow; M.L. part. adj. *nigrifluens* black flowing.

The characteristics are as described for the genus and as listed in Tables 5.26–5.28.

Colonies on Bacto-EMB (Difco) agar are dark violet with a green metallic sheen. Craters form around colonies on the polypectate medium of Hildebrand (1971).

Growth media should contain yeast extract and should be at pH 7–8.

Causes a bark necrosis of the Persian walnut (*Juglans regia*).

The mol% G + C of the DNA of one strain is 56.1 (Bd).

Type strain: ATCC 13028 (Dye, 1968).

14. **Erwinia stewartii** (Smith 1898) Dye 1963, 504.[AL] (*Pseudomonas stewarti* Smith 1898, 422.)

stew.ar'ti.i. M.L. gen. n. *stewartii* of Stewart; named after F. C. Stewart.

The characteristics are as described for the genus and as listed in Tables 5.26–5.28.

Growth slow, but better on nutrient media with a utilizable carbohydrate such as glucose or sucrose than without.

Causes a vascular wilt of corn (*Zea mays*) and some related plants, and exists in its insect vector, *Chaetocnema pulicaria*.

The mol% G + C of the DNA of two strains is 54.6 and 55.1 (Bd).

Type strain: ATCC 8199 (Approved Lists, 1980).

15. **Erwinia uredovora** (Pon, Townsend, Wessman, Schmitt and Kingsolver 1954) Dye 1963, 149.[AL] (*Xanthomonas uredovorus* Pon, Townsend, Wessman, Schmitt and Kingsolver 1954, 710).

ur.e.do'vo.ra. L. n. *uredo* blight; L. v. *voro* to devour; M.L. adj. *uredovora* blight-devouring (i.e., eats uredospores and uredia).

Craters form around colonies on polypectate gel A of Hildebrand (1971).

Attacks uredia of *Puccinia graminis* and can exist in soil.

The mol% G + C of the DNA of five strains ranges from 53.0–54.5 (Bd, T_m).

Type strain: ATCC 19321 (Sneath and Skerman, 1966).

Species Incertae Sedis

The taxonomic position of the following species is doubtful.

a. *Erwinia cancerogena* Urosevic 1966, 500.[AL]

can.cer.o'ge.na. L. n. *cancer* crab, the disease cancer; L. v. *gigno* to produce; M.L. fem. adj. *cancerogena* cancer-inducing.

Causes a canker disease of poplar (*Populus* species).

This species produces positive reactions for arginine and ornithine decarboxylase. It is probably a species of *Enterobacter*.

Type strain: NCPPB 2176 (Approved Lists, 1980).

b. *Erwinia carnegieana* Standring 1942, 310.[AL] (*Pectobacterium carnegieana* (Standring 1942) Brenner, Steigerwalt, Miklos and Fanning 1973, 205.[AL])

car.ne.gie.a'na. M.L. adj. *carnegieana* pertaining to *Carnegiea*, the name of a cactus.

In the original description *E. carnegieana* is described as, *inter alia*, a Gram-positive organism which does not ferment lactose, produces a necrotic disease of *Carnegiea gigantea* and does not attack *Opuntia* species or rot carrots. Later, Boyle (1949) with other isolates showed that the Gram reaction became nearly negative with continued culture, confirmed the lactose reaction and showed that they were not agglutinated by *E. carotovora* antiserum. Burkholder (1957) emended the description to, *inter alia*, Gram-negative with Gram-positive granules in the cells of old cultures and lactose-positive. Alcorn (1961) obtained isolates from *C. gigantea* and *Opuntia* species that would cross-infect.

Strain NCPPB 439 is Gram-negative, lactose-positive, does not rot carrots or liquefy pectate gel and produces lysine decarboxylase; two of Alcorn's isolates (NCPPB 671 and 672) are typical of *E. carotovora* (Lelliott and Graham, unpublished observations). There may therefore be two pathogens of *C. gigantea*: *E. carnegieana* and *E. carotovora*, both of which cause a similar disease.

Type strain: NCPPB 439 (ATCC 33259) (Sneath and Skerman, 1966).

Editorial Note

The type strain of *E. carnegieana* (ATCC 33259) has been identified as a typical *Klebsiella pneumoniae* (R. L. Gherna, American Type Culture Collection, personal communication).

c. *Erwinia dissolvens* (Rosen 1922) Burkholder 1948, 472.[AL] (*Pseudomonas dissolvens* Rosen 1922, 497.)

dis.sol'vens. L. part. adj. *dissolvens* dissolving.

Nonmotile. Produces large amounts of gas from many carbohydrates and decarboxylates arginine and/or lysine (Dye, 1969).

Isolated from rotting cornstalks (*Zea mays*).

This organism belongs to the genus *Enterobacter* (Waldee, 1945; Dye, 1969; Steigerwalt et al., 1976) as a new species or as a biogroup of *E. cloacae*. Its DNA is 60–80% related to DNA from *E. cloacae* and the two organisms are very similar biochemically (Steigerwalt et al., 1976).

Type strain: ATCC 23373 (Approved Lists, 1980).

d. *Erwinia nimipressuralis* Carter 1945, 423.[AL]

ni.mi.pres.su.ra'lis. L. adv. *nimis* overmuch; L. n. *pressura* pressure; M.L. adj. *nimipressuralis* with excessive pressure.

Isolated from wet wood of elms (*Ulmus* species) but its pathogenicity is doubtful.

Strains produce large amounts of gas from many carbohydrates (including lactose), decarboxylate arginine and produce lipase.

This organism belongs to the genus *Enterobacter* (Graham, 1964; Dye, 1969b; Steigerwalt et al., 1976) as a new species or as a biogroup of *E. cloacae*. Its DNA is 55–65% related to the DNA of *E. cloacae* and *Erwinia dissolvens* (see paragraph c above) (Steigerwalt et al., 1976). It is sucrose- and raffinose-negative, characteristics which separate it from *E. cloacae* (Steigerwalt et al., 1976).

Type strain: ATCC 9912 (Sneath and Skerman, 1966).

Important Notes for Users of this Edition

1. Always read both generic and species descriptions because characters listed in the generic description are not usually listed in the species descriptions.
2. Unless otherwise indicated in footnotes to tables, the meanings of symbols are as follows:
   - \+ 90% or more of strains are positive
   - − 90% or more of strains are negative
   - d 11–89% of strains are positive
   - v strain instability (*not* equivalent to "d")
   - D different reactions in different taxa (species of a genus or genera of a family)
3. All other symbols are defined in footnotes to tables.

Genus VIII. **Serratia** *Bizio 1823, 288*[AL]

PATRICK A. D. GRIMONT AND FRANCINE GRIMONT

Ser.ra'ti.a. M.L. fem noun *Serratia*, named after Serafino Serrati, an Italian physicist.

Straight rods, 0.5–0.8 μm in diameter and 0.9–2.0 μm in length, with rounded ends. Conform to the general definition of the family *Enterobacteriaceae*. Generally **motile**, by means of peritrichous flagella. Facultatively anaerobic. Colonies are most often opaque, somewhat iridescent, and either **white, pink or red in color.** Almost all strains can grow at temperatures between 10 and 36°C, at pH 5–9, and in the presence of 0–4% (w/v) NaCl. The catalase reation is strongly positive. **Acetoin is produced from pyruvate.** Reducing compound(s) are produced from gluconate. D-Glucose is fermented in the presence (and in the absence) of 0.001 M iodoacetate. Maltose, mannitol and trehalose are fermented and utilized as sole carbon sources. D-Alanine, L-alanine, 4-aminobutyrate, **caprylate**, citrate, **L-fucose**, D-glucosamine, kynurenate, L-proline, putrescine and tyrosine are utilized as sole carbon sources. Dulcitol and tagatose are neither fermented nor utilized as sole carbon sources. Butyrate and 5-amino-valerate are not utilized as sole carbon sources. **Extracellular enzymes hydrolyze DNA, lipids** (tributyrin, corn oil) **and proteins** (gelatin, casein), but not starch (in 4 days), polygalacturonic acid or pectin. Phenylalanine (and tryptophan) deaminase and thiosulfate reductase (H_2S from thiosulfate) are not produced. ***o*-Nitrophenyl-β-D-galactopyranoside (ONPG) is hydrolyzed by most strains. Chlorate is reduced** anaerobically by *Serratia* nitrate reductase (anaerobic growth does not occur with chlorate). **Growth factors are generally not required** by *Serratia* strains. The organisms **occur in the natural environment** (soil, water, plant surfaces) **or as opportunistic human pathogens.** The mol% G + C of the DNA is 52–60 (T_m, Bd).

Type species: *Serratia marcescens* Bizio 1823, 288.

Further Descriptive Information

Cells of *Serratia* rarely show a visible capsule in India ink mounts, although mucoid colonies can be observed in *S. plymuthica* and occasionally in other *Serratia* species; however, cells of *S. odorifera* possess a microcapsule which can be evidenced by the quellung reaction (capsular swelling) using *Klebsiella* anticapsule K4 or K68 sera (Richard, 1979). Polysaccharides, excreted by cells of *S. marcescens*, can be extracted from the cell surface layer or from the culture medium. These polysaccharides contain chiefly D-glucose and glucuronic acid and lower proportions of D-mannose, heptose, L-fucose and L-rhamnose (Adams and Martin, 1964; Adams and Young, 1965).

Colony diameters are ~1.5–2.0 mm after overnight growth on nutrient agar. Swarming does not occur.

Two different pigments can be produced by various *Serratia* strains: prodigiosin and pyrimine (Williams and Qadri, 1980). Prodigiosin, a nondiffusible, water-insoluble pigment bound to the cell envelope, is produced by two biogroups (A1 and A2) of *S. marcescens* and by most strains of *S. plymuthica* and *S. rubidaea*. Prodigiosin-producing colonies are totally red or show either a red center, a red margin or red sectors. The exact color given by the pigment depends upon cultural conditions (e.g. amino acids, carbohydrates, pH, inorganic ions, temperature) and may include orange, pink, red, or magenta. Prodigiosin is best produced on peptone-glycerol agar* at 20–35°C. The temperature range for pigment production is 12–36°C. Prodigiosin is not produced anaerobically. Chemically, prodigiosin is 2-methyl-3-amyl-6-methoxyprodigiosene (prodigiosene is 5-(2-pyrryl)-2,2'-dipyrrylmethene). In the cell, prodigiosin is formed by condensation of a volatile 2-methyl-3-amylpyrrol (MAP) and a nonvolatile 4-methoxy-2-2'-bipyrrole-5-carboxaldehyde (MBC). Several classes of nonpigmented mutants have been isolated that are either blocked on the MAP pathway or the MBC pathway. Syntropic pigmentation may occur when two different class mutants are grown side by side (Williams and Qadri, 1980).

Pyrimine, a water-soluble, diffusible pink pigment (Williams and Qadri, 1980), is produced by some strains of *S. marcescens* biogroup A4. Ferrous iron is required for the production of pyrimine. Pyrimine is L-2(2-pyridyl)-Δ'-pyrroline-5-carboxylic acid. When pyrimine is produced, the agar medium turns pink while the colonies are white to pinkish.

Cultures can produce two kind of odors, a fishy to urinary odor attributed to trimethylamine (mixed with some NH_3), or a musty, potato-like odor resembling that of 2-methoxy-3-isopropyl-pyrazine. The musty odor is produced by *S. odorifera*, *S. ficaria*, and a few strains of *S. rubidaea*. All other strains and species produce the fishy-urinary odor.

Several species can grow readily at 4–5°C (*S. liquefaciens*, *S. plymuthica*, *S. odorifera* and *S. ficaria*) or at 40°C (*S. marcescens* and several strains of *S. rubidaea* and *S. odorifera*); however, the temperature of 37°C is not favorable for the isolation of *S. plymuthica*. When *S. liquefaciens* and *S. plymuthica* are studied, many tests that are positive at 28–35°C give negative results at 37°C (e.g. Voges-Proskauer, decarboxylases, tetrathionate reductase tests).

A strong catalase activity, which can be evidenced with 3% (or less) H_2O_2, is produced by *Serratia* strains (Taylor and Achanzar, 1972).

There is no sodium ion requirement for growth in the genus *Serratia*; however, the optimum concentration of NaCl for growth is ~0.5% (w/v) for *S. marcescens* or 1% (w/v) for *S. rubidaea* (unpublished results). Tolerance to NaCl ranges from 5–6% (w/v) for *S. plymuthica* to 10% (w/v) for *S. rubidaea*.

In a minimal medium containing ammonium sulfate as the nitrogen source, the following compounds serve universally as sole carbon sources for all *Serratia* strains: *N*-acetylglucosamine, D-alanine, L-alanine, 4-aminobutyrate, aspartate, caproate, caprylate, citrate, D-fructose, L-fucose, fumarate, D-galactose, D-galacturonate, D-glucose, D-glucuronate, L-glutamate, L-histidine, inositol, kynurenate, L-malate, maltose, D-mannitol, D-mannose, L-proline, putrescine, pyruvate, D-ribose, L-serine, succinate, trehalose and L-tyrosine. Most strains of all species can utilize acetate, caprate, D-glucosamine, glycerate, glycerol, lactate, phenylacetate, salicin and L-tryptophan. The following compounds are never utilized as sole carbon sources: acetamide, adipate, 4-aminobenzoate, DL-5-aminovalerate, α-amylamine, azelate, benzylamine, butanol, butylamine, butyrate, citraconate, DL-citrulline, creatine, dulcitol, ethylene glycol, D-fucose, glutarate, glycolate, L-isoleucine, isophthalate, isopropanol, isovalerate, levulinate, L-leucine, D-mandelate, L-mandelate, mesaconate, methanol, methylamine, DL-norleucine, oxalate, pantothenate, phenol, phthalate, pimelate, propanol, propylene glycol, sebacate, sorbose, spermine, suberate, terephthalate, testosterone, tryptamine, D-tryptophan, turanose, uracil, urea, L-valine, and L-xylose (Grimont et al., 1977b, 1978a, 1979b).

Characteristic extracellular enzymes are produced. All species recognized herein can hydrolyze DNA, gelatin, soluble casein, tributyrin, and corn oil. Only rare strains fail to produce one or more of these extracellular enzymes. All species, except *S. odorifera*, can hydrolyse Tween 80. Chitin is hydrolyzed by all species except *S. rubidaea* and *S. odorifera*. Lecithin is also hydrolyzed by many strains. Spot-inoculated starch agar,† incubated for 4 days and then flooded with Lugol's iodine, shows no zone of clearing (Grimont et al., 1977b); however, longer incubation (6–14 days) may allow detection of some amylase-producing strains (M. Popoff, personal communication).

---

* Peptone glycerol agar: Bacto-peptone (Difco), 5.0 g; glycerol, 10.0 ml; Bacto-agar (Difco), 20.0 g; distilled water, 1000 ml.

† Starch agar: nutrient agar containing 0.5% (w/v) soluble starch.

A red-pigmented *S. marcescens* has been found to produce a carboxymethyl cellulase (Thayer, 1978). Depolymerization of a carboxymethyl cellulose gel is faster with *S. marcescens*, *S. rubidaea*, and *S. liquefaciens* than with *S. odorifera*, *S. ficaria* and *S. plymuthica* (unpublished results).

Up to 11 proteinases have been revealed by agar gel electrophoresis. Each strain produces one to four different proteinases. Different species have different proteinase patterns (Grimont et al., 1977a). Isoelectric points of the 11 proteinases are between pH 3.6 and pH 6.0 (Grimont and Grimont, 1978a).

Fructose, maltose, D-mannitol, D-mannose, ribose, and trehalose are fermented by all strains. Most strains ferment glycerol and *myo*-inositol. Fermentation of D-glucose is not prevented by 0.001 M iodoacetate (Grimont et al., 1977b, 1978a, 1979b), an inhibitor of the Embden-Meyerhof-Parnas glycolytic pathway and other enzymic reactions. *Serratia* species can produce gluconate-6-phosphate dehydrase and 2-keto-3-deoxygluconate-6-phosphate aldolase (Kersters and De Ley, 1968), which are the characteristic enzymes of the Entner-Doudoroff pathway. Under aerobic conditions, 2-ketogluconic acid is produced from D-glucose (Misenheimer et al., 1965). A reducing compound (probably 2-ketogluconate) is also produced from gluconate by all species (Grimont et al., 1977b, 1978a, 1979b).

The Voges-Proskauer (VP) test, when done on a 3-day-old culture in Clark-Lubs medium, is negative for 40% of the strains of *S. plymuthica*, although acetoin can be detected after incubation for 18 h by use of a sensitive method (Richard, 1972). These strains which are VP-negative after 3 days incubation, can utilize 2,3-butanediol as a sole carbon source (Grimont et al., 1977b). *Serratia* strains that cannot produce acetoin from pyruvate (under any experimental conditions) are very rare. A tiny gas bubble is commonly produced by *S. marcescens* in a peptone-water-glucose medium with Durham tube. *S. plymuthica* and *S. liquefaciens* produce a larger amount of gas. The end products of glucose fermentation by *S. marcescens* are 2,3-butanediol, ethanol, formate, lactate, succinate and CO_2, with small amounts of acetate, acetoin and glycerol and very little or no H_2 (Neish et al., 1948; White and Starr, 1971). The end products yielded by *S. plymuthica* are 2,3-butanediol, ethanol, lactate, succinate, CO_2, H_2 and small amounts of formate, acetate, acetoin, and glycerol (Neish et al., 1948). The 2,3-butanediol produced by *S. marcescens* is mostly a *meso*-isomer, whereas *S. plymuthica* is unique in producing a *levo*-rotatory 2,3-butanediol (Neish et al., 1948).

Transduction systems have been described in *S. marcescens* (Kaplan and Brendel, 1969; Matsumoto et al., 1973). The earliest genetic transfer described in *S. marcescens* (Belser and Bunting, 1956) is also suggestive of a transduction mechanism.

Lactose plasmids have been demonstrated in *S. liquefaciens* (Le Minor et al., 1974) and in *S. marcescens* (C. Coynault, personal communication). Antibiotic resistance plasmids of incompatibility groups H_2, C, M, P, W, and F_{II} have been identified in *S. marcescens*. Plasmids of groups M and N have been found in *S. liquefaciens* (Hedges, 1980).

Bacteriophages active on *Serratia* are easily found in river water or sewage. Phages that are active on one species of *Serratia* are usually active on strains of other species of that genus but rarely on strains of other genera (Grimont and Grimont, 1978a). Lyosgeny is very common in *Serratia* species (Prinsloo, 1966). Several phage typing systems have been studied (Pillich et al., 1964; Hamilton and Brown, 1972; Farmer, 1975; F. Grimont, Doctorate in Pharmacy thesis, University of Bordeaux II, 1977).

Bacteriocins produced by *Serratia* are of two kinds: (a) a trypsin-resistant, acid-sensitive (pH 2) structure (Hamon and Péron, 1961) called "group A bacteriocin" by Prinsloo (1966) and later found by electron microscopy to resemble phage tails (Traub, 1972); and (b) a trypsin-sensitive, acid-resistant protein (Hamon and Péron, 1961) called "group B bacteriocin" by Prinsloo (1966). Bacteriocins produced by one species of *Serratia* frequently cross-react with other species of this same genus. *Serratia* bacteriocins are also frequently active on *Escherichia coli* K12. *S. marcescens* strains produce group A and/or group B bacteriocins. *S. rubidaea* strains produce only group A bacteriocins. *S. liquefaciens* and *S. ficaria* produce only group B bacteriocins. *S. odorifera* produce neither group A or group B bacteriocins (Hamon and Péron, 1979; Y. Hamon, personal communication). Bacteriocin typing can be used for epidemiological purposes (Traub, 1980).

The antigenic structure of only one species (*S. marcescens*) has been detailed. The present scheme consists of 21 somatic antigens (01 to 021) and 25 flagellar antigens (H1 to H25) (Edwards and Ewing, 1972; Le Minor and Pigache, 1978; Traub and Fukushima, 1979; Le Minor and Sauvageot-Pigache, 1981).

Subdivision of antigens 05 (into 05a, 05b, 05c), 010 (into 010a, 010b), and 016 (into 016a, 016b, 016c, 016d) has been proposed (Le Minor and Sauvageot-Pigache, 1981). Cross-reactions between factors 06 and 014 are very extensive and the distinction between these two factors does not seem worthwhile. H antigens are monophasic in *S. marcescens*.

Resistance to cephalothin, colistin, and polymyxin (with respect to achievable serum levels of antibiotics) is very frequent in the genus and almost constant in *S. marcescens*. With the antibiotic disk method, a zone phenomenon develops around disks impregnated with colistin and polymyxin: the inhibition zone contains colonies close to the disk. However, this zone phenomenon is not restricted only to *Serratia*. Resistance to tetracycline and ampicillin is very frequent in *S. marcescens* and rare in other *Serratia* species. Plasmid-determined resistance to aminoglycoside antibiotics, carbenicillin, chloramphenicol, trimethoprim, sulfonamides and mercury ions can be found in clinical strains of *S. marcescens*. Resistance to cetyltrimethylammonium chloride (1.5 mg/ml) and thallous acetate (0.8 mg/ml) is very frequent (Grimont et al., 1977b). Of all the *Serratia* species, *S. marcescens* is the most resistant to antibiotics, antiseptics, and metal ions; *S. plymuthica* is the least resistant to these antimicrobials.

A typical hypersensitivity reaction is produced by inoculation of plants such as tobacco and king protea with *Serratia* (Lakso and Starr, 1970; Grimont et al. 1978b). *S. proteamaculans* was isolated from a leaf spot disease of *Protea cynaroides* (Paine and Stanfield, 1919) and *S. marcescens* (under the name *Erwinia amylovora* var. *alfalfae*) was isolated from a root disease of alfalfa (Shinde and Lukezic, 1974).

S. marcescens and *S. liquefaciens* are potential pathogens for insects. Pathogenicity is correlated with the production of lecithinase, proteinase and chitinase (Lysenko, 1974; Lysenko, 1976; Kaska, 1976).

Mastitis in cows and other animal infections have been associated with *Serratia* species (Grimont and Grimont, 1978a). Pathogenicity in experimental animals is of the type expected of a Gram-negative bacterium. Experimental depression of phagocytic cell number or function in animals enhances susceptibility to *Serratia* infections (Simberkoff, 1980).

S. marcescens is a prominent opportunistic pathogen for hospitalized human patients. Other *Serratia* species can be involved in bacteremia, especially when accidentally injected into the body (contaminated perfusion or irrigation liquid). They can also be isolated from sputum without having clinical significance (Grimont and Grimont, 1978a).

Serratia species occur on plants, in the digestive tract of rodents (unpublished data), and in soil and water. *S. ficaria* is especially associated with the fig/fig-wasp ecosystem (Grimont et al., 1979b).

Enrichment and Isolation Procedures

Fecal samples (diluted with distilled water) or plant material washings are inoculated onto caprylate-thallous (CT) agar* (Starr et al.,

* Caprylate-thallous (CT) agar: autoclaved solutions A and B are mixed aseptically in equal volumes and the mixture is poured into Petri dishes. *Solution A*: $CaCl_2 \cdot 2H_2O$, 0.0147 g; $MgSO_4 \cdot 7H_2O$, 0.123 g; KH_2PO_4, 0.68 g; K_2HPO_4, 2.61 g; trace element solution (see below), 10 ml; caprylic acid, 1.1 ml; yeast extract (Difco), 5% (w/v) solution, 2.0 ml; thallous sulfate, 0.25 g; distilled water to 500 ml. Adjust the pH to 7.2. *Trace element solution*: distilled water, 1000 ml; H_3PO_4, 1.96 g; $FeSO_4 \cdot 7H_2O$, 0.0556 g; $ZnSO_4 \cdot 4H_2O$, 0.0287 g; $MnSO_4 \cdot 4H_2O$, 0.0223 g; $CuSO_4 \cdot 5H_2O$, 0.025 g; $Co(NO_3)_2 \cdot 6H_2O$, 0.003 g; H_3BO_3, 0.0062 g. This solution keeps well at 4°C for at least 2 years. *Solution B*: NaCl, 7.0 g; $(NH_4)_2SO_4$, 1.0 g; agar

1976). After 2–5 days, the growth is removed by scraping and tested for deoxyribonuclease (DNase) activity. DNase-positive cultures are then purified by streaking a nonselective medium (e.g. tryptic soy agar). Different colonial types are tested for DNase, and DNase-positive isolates are then thoroughly characterized and identified. This procedure allows isolation of all *Serratia* species as defined in this chapter. *Providencia*, *Acinetobacter* and fluorescent *Pseudomonas* strains can grow on CT agar when samples contain large numbers of these organisms. Other selective media based on DNase production and antibiotic resistance have been proposed (Farmer et al., 1973; Cate, 1972; Berkowitz and Lee, 1973). These antibiotic-containing media are efficient for the isolation of *S. marcescens* but may not be as reliable for more sensitive species (e.g. *S. plymuthica*).

Maintenance Procedures

For short term preservation (several months), heavy suspension of bacteria in sterile distilled water are made from bacterial growth scraped with a platinium loop from a nutrient agar slant. The suspensions are stored at room temperature.

For longer preservation (several years), screw-capped tubes containing semisolid nutrient agar† are stab-inoculated. After overnight growth at 30°C, the tubes are tightly closed and kept at room temperature in the dark. Maintenance failure may occur if the tube is not protected from desiccation by a rubber seal in the screw cap. Rubber corks dipped in melted paraffin wax may be preferred in place of screw caps.

For long term preservation (over 5 years), freeze-drying is preferred.

Procedures for Testing Special Characters

Carbon source utilization test. The defined medium M70 of Véron (1975) without yeast extract is made by mixing equal volumes of freshly autoclaved solutions A and B. *Solution A* contains $CaCl_2 \cdot 2H_2O$, 0.0147 g; $MgSO_4 \cdot 7H_2O$, 0.123 g; KH_2PO_4, 0.680 g; K_2HPO_4, 2.610 g; trace element solution (same as for CT agar), 10 ml; and distilled water to 500 ml. Adjust to pH 7.2 with NaOH. *Solution B* is the same as in CT agar. The carbon source solution (1 g of carbon source in ~5 ml of distilled water; salts or hydrated molecules are weighted so as to have 1 g of the organic ion) is adjusted to pH 7, sterilized by filtration, and added to the complete medium while the latter is still hot and molten. The medium is then dispensed into divided Petri dishes ("Replidish" Sterilin, Teddington, England) with 3 ml per well. After drying overnight at 37°C, the plates are inoculated with a multipoint inoculator (Denley). Inoculated plates are incubated at 30°C and examined for growth every other day for 12 days. If the room temperature is about 20–25°C, the plates can be removed from the incubator after 4 days and kept at room temperature; this can be useful if incubators are crowded with cultures or materials that can exhale volatile carbon sources. Only unequivocal growth should be recorded as positive.

Voges-Proskauer test (Richard's modification). Clark and Lubs medium (BBL) is dispensed in large 22 × 215 mm tubes (0.5 ml per tube) and inoculated with 0.05 ml of a heavy bacterial suspension in distilled water. After incubation at 30°C for 18 h, 0.5 ml of α-naphthol solution (6% w/v alcoholic solution) and 0.5 ml of 4 M NaOH are added. The tubes are shaken, heated briefly in a Bunsen flame, and examined for a red color (Richard, 1972).

Tetrathionate reduction. The medium of Le Minor et al. (1970) contains: peptone (Difco), 10.0 g; NaCl, 5.0 g; $K_2S_4O_6$, 5.0 g; bromthymol blue (0.2% aqueous solution), 25 ml; and distilled water to 1 liter. Adjust the pH to 7.4, sterilize by filtration, and dispense into 12 × 120 mm tubes (4 ml per tube). The size of the tubes (for a rather limited aeration) is critical. Inoculated tubes are incubated at 30°C for 24 h and examined for a yellow color (tetrathionate reduction).

β-Xylosidase. Paper disks (0.5 cm) are loaded with 0.1 ml of a 2% (w/v) aqueous solution of *p*-nitrophenyl-β-D-xylopyranoside and kept dry in a tightly-capped flask at 4°C. the test is performed exactly like the β-galactosidase test, but with *p*-nitrophenyl-β-D-xylopyranoside disks in place of ONPG disks (Brisou et al., 1972).

H-Immobilization test. The motility of each isolate to be typed must be enhanced by passage through a 0.3% semisolid agar U-tube.

The following autoclaved semisolid medium is dispensed in 2.0-ml volumes into small (92 × 13 mm) screw-capped tubes: tryptic peptone, 20.0 g; D-mannitol, 2.0 g; KNO_3, 1.5 g; phenol red solution (1%), 4 ml; agar, 4.5 g; distilled water, 1000 ml; pH 7.4. The tubes of semisolid medium are melted (boiling water bath), cooled to 50°C in a water bath, supplemented with 0.05 ml of each serum dilution under sterile conditions, and allowed to gel.

Tubes with serum dilutions (and control tubes without serum) are stab-inoculated with a highly motile culture. After overnight incubation, tubes are examined for immobilization. This H-immobilization test is very specific and much easier to perform than the classical H-agglutination (Le Minor and Pigache, 1977).

Differentiation of the genus **Serratia** *from other genera*

Table 5.30 provides the primary characteristics that can be used to differentiate the genus *Serratia* (as defined in this chapter) from biochemically similar taxa.

Taxonomic Comments

A number of changes have been made since the eighth edition of the *Manual* in which it was indicated that the genus *Serratia* was composed of only one species, *S. marcescens* (the type species).

Transfer of *Enterobacter liquefaciens* to the genus *Serratia* was first proposed by Barbe (Doctor in Pharmacy thesis, University of Marseille, France, 1969) and supported by studies on bacteriocin cross-reactions between *S. marcescens* and *E. liquefaciens* (Hamon et al., 1970). Valid publication of the new combination *S. liquefaciens* followed a numerical taxonomy study (Bascomb et al., 1971).

A phenon named "biotype 2" (Bascomb et al., 1971) and "phenon B" (Grimont and Dulong de Rosnay, 1972) was thought identical to *Bacterium rubidaeum* Stapp 1940 and named *S. rubidaea* (Ewing et al., 1973). The same phenon was also identified as *S. marinorubra* Zobell and Upham 1944 (Grimont et al., 1977, 39). *S. rubidaea* and *S. marinorubra* were based on different type strains (ATCC 27593 and ATCC 27614, respectively). The Approved Lists of Bacterial Names, however, give both names *S. rubidaea* and *S. marinorubra* with the same type strain (viz. ATCC 27614, the type strain of *S. rubidaea*). Hence, both names, which were subjective synonyms, are now objective synonyms and redundant. To avoid further confusion, the name *S. rubidaea* (Stapp) Ewing et al. should now be used exclusively to designate the same (*S. rubidaea-S. marinorubra*) taxon.

The ancient species *S. plymuthica* (Lehman and Neumann 1896) Breed, Murray, and Hitchens 1948 was shown to be a valid species by numerical taxonomy (Grimont et al., 1977b) and by DNA/DNA hybridization (Grimont et al., 1978a).

Recently, two new species, *S. odorifera* Grimont et al., 1978, 453 and *S. ficaria* Grimont, Grimont, and Starr 1981 were defined by DNA relatedness, carbon source utilization tests, and by standard biochemical tests.

DNA relatedness studies have shown that *S. marcescens*, *S. plymuthica*, *S. rubidaea*, *S. odorifera*, and *S. ficaria* are homogeneous and discrete genospecies (Steigerwalt et al., 1976; Grimont et al., 1978a;

(Difco), 15.0 g; distilled water to 500 ml. Adjust the pH to 7.2. The complete medium keeps well at 4°C. It should not be remelted once it has solidified.

† Semisolid nutrient agar: meat extract (Liebig), 3.0 g; yeast extract (Difco), 10.0 g; agar (BBL or Difco), 7.5 g; distilled water to 1000 ml. Adjust pH to 7.4.

Table 5.30.
Differential characteristics of the genus **Serratia** *and other biochemically similar taxa*[a]

Characteristics	*Serratia*	"*Serratia*" *fonticola*	*Erwinia herbicola-Enterobacter agglomerans* group	*Enterobacter cloacae*	Pectinolytic *Erwinia*[b]	*Klebsiella*[c]
Carbon source utilization test:						
4-Aminobutyrate	+	−	+	d	−	D
5-Aminovalerate	−	−	−	−	−	D
Arginine	−	−	−	−	−	+
Caprate	+	−	−	−	−	−
Caproate	+	−	−	−	−	−
Caprylate	+	−	−	−	−	−
D-Dulcitol	−	+	−	−	−	−
L-Fucose	+	−	−	−	−	+
Pelargonate	D	−	−	−	−	−
Tagatose	−	+	−	−	−	D
Tyrosine	+		−	−	−	D
Voges-Proskauer test	+	−	+	+	+	+
Gelatin, hydrolyzed	+	−	D	d	D	D
Tributyrin, hydrolyzed	+		−	−	−	−
Deoxyribonuclease	+	−	−	−	D	−
Gluconate test[d]	+	+	−	+	−	+
Iodoacetate test[e]	+	+	+	+	−	+
Mol% G + C of DNA	52–60	48.8–52.5	53.5–56	53	51–54	53.8–57

[a] Symbols: see standard definitions.
[b] Including *Erwinia carotovora*, *E. atroseptica* and *E. chrysanthemi*.
[c] Including *Klebsiella pneumoniae* and *K. mobilis* (*Enterobacter aerogenes*).
[d] Production of reducing compound(s) from gluconate.
[e] Production of acid from glucose in the presence of 0.001 M iodoacetate.

1979b). *S. liquefaciens* is heterogeneous (Steigerwalt et al., 1976) and is probably composed of several genospecies. One biovar (C1c) of *S. liquefaciens* was identified as *Erwinia proteamaculans* (Paine and Stansfield, 1919) Dye 1966 and renamed *S. proteamaculans* (Grimont et al., 1978, 503). Reexamination of DNA relatedness in *S. liquefaciens* disclosed at least three genospecies: *S. liquefaciens* sensu stricto, *S. proteamaculans* (Grimont et al., 1981), and a third group containing strain ATCC 14460 and named *S. grimesii* (Grimont et al., 1982 a, b).

A group of strains called "*Citrobacter* lysine^{+}" or "*Citrobacter*-like" was found to be related significantly to the genus *Serratia* in DNA/DNA hybridization studies (Crosa et al., 1974). This genospecies has recently been named *Serratia fonticola* Gavini et al., 1979; however, a difficulty is that *S. fonticola* does not have the key characteristics of the genus *Serratia* (Table 5.31). Furthermore, *Serratia* phages which are active on strains of any *Serratia* species (as defined herein) have been found to be inactive on all *S. fonticola* strains tested (unpublished data). Bacteriocins from *Serratia* are also inactive on *S. fonticola* (Hamon, personal communication). In this chapter, *S. fonticola* is considered to be a *species incertae sedis* pending further study.

All molecular approaches to taxonomy (e.g. genome size, DNA relatedness, immunologic cross-reactions between iso-functional enzymes, physical properties and regulation of enzymes, amino acid sequences of enzymes) support the distinction of the genus *Serratia* from the other members of the family *Enterobacteriaceae* (reviewed by Grimont and Grimont, 1978a).

Further Reading

Edwards, P.R. and W.H. Ewing. 1972. Identification of *Enterobacteriaceae*, 3rd Ed., Burgess Publishing, Minneapolis, Minn.
Grimont, P.A.D. and F. Grimont. 1978. The genus *Serratia*. Annu. Rev. Microbiol. *32*: 221–248.
Grimont, P.A.D. and F. Grimont. 1981. The genus *Serratia*. In Starr, Stolp, Trüper, Balows, and Schlegel (Editors), *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria*, Springer-Verlag, New York, pp. 1187–1203.
Grimont, P.A.D., F. Grimont, H.L.C. Dulong de Rosnay, and P.H.A. Sneath. 1977. Taxonomy of the genus *Serratia*. J. Gen. Microbiol. *98*: 39–66.
Von Graevenitz, A. and S.J. Rubin. 1980. The genus *Serratia*, CRC Press, Boca Raton, Fla.

Differentiation and characteristics of the species of the genus **Serratia**

The differential characteristics of the species of *Serratia* are indicated in Table 5.31. Other characteristics of the species are listed in Table 5.32.

List of the species of the genus **Serratia**

1. **Serratia marcescens** Bizio 1823, 288.[AL]

mar.ces'cens. M.L. v. *marcesco* to fade; L. part. adj. *marcescens* fading away.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin or pyrimine can be produced.

Physiological and nutritional characteristics are presented in Tables 5.31 and 5.32.

A biotyping system based on pigment production, tetrathionate reduction, and utilization of *meso*-erythritol, trigonelline, quinate, benzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, and DL-carnitine as sole

Table 5.31.
Characteristics differentiating the species of the genus **Serratia**[a]

Characteristics	1. *S. marcescens*	2. *S. liquefaciens*	3. *S. plymuthica*	4. *S. rubidaea*	5. *S. odorifera*	6. *S. ficaria*
Growth on[b] and acid production from:						
L-Arabinose, D-melibiose, D-xylose	−	+	+	+	+	+
Xylitol	+	−	−	−	+	+
D-Melezitose	−	+	+	db	−	+
L-Rhamnose	−	db	−	−	+	+
D-Sorbitol	+	+	db	−	+	+
D-Arabitol	−	−	−	+	−	+
Growth on:						
Betaine	−	−	db	+	−	−
Nicotinate	+	+	+	−	+	+
D-Tartrate	−	−	−	db	+	−
Trigonelline	db	−	−	+	+	+
Prodigiosin production	db	−	d	+	−	−
Musty odor	−	−	−	d	+	+
Good growth at 4°C	−	+	+	−	+	+
Indole production	−	−	−	−	+	−
Tetrathionate reduction	db	+	−	−	−	−
Lysine decarboxylase (Møller)	+	+	−	db	+	−
Ornithine decarboxylase (Møller)	+	+	−	−	db	−
β-Xylosidase	−	−	db	+	+	v
Tween 80 hydrolysis	+	+	+	+	−	+
Chitin hydrolysis	+	d	+	−	−	+
Gas from glucose	−	+	d	−	−	−

[a] Data from Grimont et al. (1977, 1978, 1979). For symbols see standard definitions; also db, test differentiates biovars.
[b] Utilization as sole carbon source.

Table 5.32.
Other characteristics of the species of the genus **Serratia**[a]

Characteristic	1. *S. marcescens*	2. *S. liquefaciens*	3. *S. plymuthica*	4. *S. rubidaea*	5. *S. odorifera*	6. *S. ficaria*
Growth on[b]:						
Acetate	+	d	d	d		+
trans-Aconitate	+	db[c]	d	+	+	+
Adonitol	+	−	−	+	+	+
β-Alanine	+	d	d	−		
Anthranilate	d	−	−	d		
DL-Arginine	−	−	−	d		
Benzoate	db	db	d	+	−	+
Benzylformate	−	−	d	d		
2,3-Butanediol	−	−	d	−		
Caprate	+	+	d	+		
Caproate	+	+	d	+		
Caprylate	+	+	+	+	+	+
DL-Carnitine	d	d	−	−	−	−
D-Cellobiose	−	−	+	+	+	+
meso-Erythritol	db	db	−	+	db	+
Ethanol	d	d	d	d		
D-Glucosamine	+	+	d	+	+	+
Glycerate	+	+	d	+		
Glycerol	+	+	d	+		+
Heptanoate	+	−	d	−		
Hippurate	d	−	−	d		
Histamine	−	−	−	d	−	−
3-Hydroxybenzoate	db	−	−	−	−	−
4-Hydroxybenzoate	db	d	d	−	−	d
3-Hydroxybutyrate	d	−	−	−		
Inulin	−	−	d	−		
α-Ketoglutarate	d	+	d	+		
Lactate	+	+	d	+		
D-Lactose	−	d	+	+	+	d
D-Malate	d	db	d	d		

Table 5.32.—*continued*

Characteristics	1. *S. marcescens*	2. *S. liquefaciens*	3. *S. plymuthica*	4. *S. rubidaea*	5. *S. odorifera*	6. *S. ficaria*
α-Methylglucoside	−	+	d	+	−	+
Mucate	−	−	d	+	+	+
L-Ornithine	d	d	−	−	db	−
Pelargonate	+	d	d	+		
Phenylacetate	+	+	d	+		
L-Phenylanine	d	d	d	+		
Propionate	+	−	d	−		
Quinate	db	db	+	+	−	+
Raffinose	−	+	+	+	db	+
Salicin	+	db	+	+		
Sarcosine	−	−	d	+		
Sucrose	+	+	+	+	db	+
L-Tartrate	−	−	−	d		
meso-Tartrate	d	db	−	−		
L-Tryptophan	d	d	d	+		
Valerate	d	−	−	−		
Growth at 37°C	+	+	d	+	+	+
Growth at 40°C	+	−	−	d	+	
Growth in NaCl:						
7% (w/v)	+	d	d	+		
8.5% (w/v)	d	d	−	+		
10% (w/v)	−	−	−	d		
Tetrathionate reduced	db	+	−	−	−	−
Methyl red test	−	d	d	−	+	−
H_2S from cysteine	+	+	+	d		+
Arginine decarboxylase (Møller)	−	db	−	−	−	−
Malonate test	−	−	−	db	−	−
Tween 40 hydrolysis	+	+	+	+	+	+
Tween 60 hydrolysis	+	+	+	+	d	+
Lecithinase (turbidity)	+	d	d	d		
Esculin hydrolysis	+	db	+	+	+	+
Growth on colistimethate (10 and 100 μg/ml)	+	+	d	d		
Acid produced from:						
Adonitol	v	−	−	+	v	+
myo-Inositol	d	+	d	d	+	+
Lactose	−	−	d	+	+	d
Raffinose	−	+	+	+	db	+
Salicin	+	db	+	+	+	+
Sucrose	+	+	+	+	db	+

[a] Data from Grimont et al. (1977b, 1978a, 1979b). For symbols see standard definitions; also db, test differentiates biovars.
[b] Carbon source utilization tests.

carbon sources, has been described (Grimont and Grimont, 1978b). Groups of biovars (called biogroups) (Table 5.33) correspond to definite, non-overlapping sets of serovars (Table 5.34) (Grimont et al., 1979a).

Nonpigmented biogroups A3 and A4 are ubiquitous. Nonpigmented biogroups A5/8 and TCT are almost confined to hospitalized patients. Pigmented biogroups A1 and A2/6 are found in the natural environment and occasionally in human patients.

The mol% G + C of the DNA is 57.5 to 60 (T_m, Bd).

Type strain: ATCC 13880.

2. **Serratia liquefaciens** (Grimes and Hennerty 1931) Bascomb, Lapage, Willcox and Curtis 1971, 293.^{AL} (*Aerobacter liquefaciens* Grimes and Hennerty 1931, 93).

li.que.fa'ciens. M.L. part. adj. *liquefaciens* dissolving.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is not produced.

Physiological and nutritional characteristics are presented in Tables 5.31 and 5.32.

Several biovars can be recognized (Table 5.35) Grimont et al., 1977b: and unpublished data). The present species *S. liquefaciens* is formed of at least three genospecies: one corresponds to biovar Clab (including the type strain of *S. liquefaciens*); another corresponds to biovars Clc (including the type strain of *S. proteamaculans*), EB, RB and RQ; and a third one corresponds to biovars Cld and Adc.

S. liquefaciens is the most prevalent *Serratia* species in the natural environment (plants, digestive tract of rodents). Occasionally encountered as an opportunistic pathogen.

The mol% G + C of the DNA is 53 to 54 (T_m, Bd).

Type strain: ATCC 27592.

The present species is biochemically and genetically heterogeneous. Splitting the present species into three species, *S. liquefaciens* sensu stricto, *S. proteamaculans*, and a new species, *S. grimesii*, can be anticipated.

3. **Serratia plymuthica** (Lehmann and Neumann 1896) Breed, Murray and Hitchens 1948, 481. (*Bacterium plymuthicum* (sic) Leh-

Table 5.33.
Identification of **S. marcescens** *biogroups and biovars*[a]

Characteristics	Biogroup						
	A1	A2/6	A3	A4	A5/8	TCT	TC
Growth on[b]:							
meso-Erythritol	+	+	+	+	−	−	−
Benzoate and hippurate	+	−	−	−	−	−	−
Quinate and 4-hydroxybenzoate	−	db[c,d]	−	db[e]	+	−	−
3-Hydroxybenzoate	−	−	db[f]	−	db[g]	−	−
Trigonelline	−	db[h]	db[i]	−	+	+	−
DL-Carnitine	db[j]	d	d	+	db[k]	db[l]	+
Tetrathionate reduction	+	+	+	−	+	+	+
Prodigiosin production	+	+	−	−	−	−	−

[a] For symbols see standard definitions.
[b] Carbon source utilization test.
[c] db, test differentiates biovars.
[d] Positive for biovars A6, negative for A2a and A2b.
[e] Positive for biovar. A4a, negative for A4b.
[f] Positive for biovars A3a and A3b, negative for A3c and A3d.
[g] Positive for biovar A8b, negative for A5 and A8a.
[h] Positive for biovar A2b, negative for A2a and A6.
[i] Positive for biovars A3b and A3d, negative for A3a and A3c.
[j] Positive for biovar A1a, negative for A1b.
[k] Positive for biovar A5, negative for A8a and A8b.
[l] Positive for biovar TCT, negative for biovar TT.

Table 5.34.
Correspondence between serovars and biogroups in **S. marcescens**[a]

Biogroup	O:H Serovars[b]
A1	*5:2*, 5:3, 5:13, 5:23, 10:6, 10:13
A2/6	6,14:2, 6,14:3, 6,14:8, 6,14:9, 6,14:10, 6,14:13, 8:3, 13:5
A3	3:5, 3:11, 4:9, 4:18, 5:6; 5:15, 6,14:5, 6,14:6, 6,14:20, 9:11, 9:17, 12:5, 12:9, 12:11, 12:17, 12:20, 13:11, *13:17*, 15:3, 15:5, 15:8, 15:9, 17:4, 18:21
A4	1:1, 1:4, 2:1, 2:8, 3:1, 4:1, 4:4, 5:1, 5:6, 5:8, 5:24, 9:1, 13:1, 13:13
A5/8	3:12, 3,21:12, 4:12, 5:4, 6,14:4, 6,14:12, 8:12, 15:12, 21:12
TCT	*1:7*, 2:7, *4:7*, 5:7, 5:19, 7:23, 11:4, 13:7, 13:12, 16:19, *18:9*, 18:16, 19:14
TC	10:8, 20:12

[a] Data from Grimont et al. (1979a), and unpublished data.
[b] Serovars for which exceptions to the correspondence occur are in italics.

mann and Neumann 1896, 264,) *Note: S. plymuthica* is cited on the Approved Lists of Names as *Serratia plymuthica* (Dyar 1895) Bergey, Harrison, Breed, Hammer and Huntoon 1923, 88. This is incorrect, for reasons discussed by Grimont et al. (1977b).

ply. mu'thi.ca. M.L. adj. *plymuthica* pertaining to Plymouth.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is produced by most strains.

Physiological and nutritional characteristics are presented in Tables 5.31 and 5.32.

Most *S. plymuthica* strains studied were isolated from fresh water. Very rarely found in human sputum. No human infection reported.

The mol% G + C of the DNA is 53.5–56.5 (T_m).

Type strain: ATCC 183.

4. **Serratia rubidaea** (Stapp 1940) Ewing, Davis, Fife and Lessel 1973, 224.[AL] (*Bacterium rubidaeum* Stapp 1940, 259; *Serratia marinorubra* Zobell and Upham 1944, 255.)

Table 5.35.
Identification of **S. liquefaciens** *biovars*[a]

Characteristics	*S. liquefaciens* biovars						
	Clab	Clc	Cld	EB	RB	RQ	Adc
Growth on[b]:							
trans-Aconitate	−	+	−	+	−	+	+
Adonitol	−	−	−	+	−	−	−
Benzoate	−	−	+	+	+	−	−
meso-Erythritol	−	−	−	+	−	−	−
D-Malate	+	−	d	−	−	d	d
Quinate	−	−	−	−	−	+	−
L-Rhamnose	−	−	−	−	+	d	−
meso-Tartrate	+	−	−	−	−	d	−
Arginine decarboxylase (Møller)	−	−	+	−	−	−	+
Tetrathionate reduction	+	+	+	+	+	d	+
Esculin hydrolysis	+	+	+	+	+	−	+

[a] For symbols see standard definitions.
[b] Carbon source utilization tests.

ru.bi'dae.a. from the Latin name *Rubus idaeus* (raspberry), contracted and made to agree in gender with *Serratia*.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is produced by most strains.

Physiological and nutritional characteristics are presented in Tables 5.32 and 5.33.

S. rubidaea strains are rarely isolated, both in the natural environment and in human patients. May be found in ripe coconuts (P. A. D. Grimont, F. Grimont and M. P. Starr, unpublished data).

The mol% G + C of the DNA is 53.5–58.3 (T_m).

Type strain: ATCC 27593.

5. **Serratia odorifera** Grimont, Grimont, Richard, Davis, Steigerwalt and Brenner 1978, 461.[AL]

o.do.ri.fe'.ra. M.L. fem. adj. *odorifera*, bringing odors, fragrant.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is not produced. Cultures give off a musty, potato-like odor.

Physiological and nutritional characteristics are presented in Tables 5.31 and 5.32.

The capsular antigen reacts with *Klebsiella* antisera K4 or K68.

Rare opportunistic pathogen. Occasionally isolated from plants or food.

The mol% G + C of the DNA is 54.6 (T_m).

Type strain: ATCC 33077.

6. **Serratia ficaria** Grimont, Grimont and Starr 1981, 216.[VP] (Effective publication: Grimont, Grimont and Starr 1979, 282.)

fi.ca'ri.a. M.L. fem. adj. *ficaria* of figs.

The cell morphology and colonial morphology are as given for the genus. Prodigiosin is not produced. Cultures give off a musty, potato-like odor.

Physiological and nutritional characteristics are presented in Tables 5.31 and 5.32.

Associated with the fig/fig-wasp biological cycle. Occasionally found on plants other than fig trees.

The mol% G + C of the DNA is 59.6 (T_m).

Type strain: ATCC 33105.

Species Incertae Sedis

a. *Serratia fonticola* Gavini, Ferragut, Izard, Trinel, Leclerc, Lefebvre and Mossel 1979, 98.[AL]

fon.ti'co.la. M.L. n. *fons, fontis* spring, fountain; L. suffix *-cola* dweller; M. L. noun *fonticola* spring-dweller.

Rod-shaped cells, described as being 0.5 × 30 μm; the latter value,

however, is probably a misprint. Gram-negative. Motile by peritrichous flagella.

Conform to the definition of the family *Enterobacteriaceae*, do not conform to the present definition of the genus *Serratia*.

Growth occurs between 4°C and 37°C. No growth at 41°C.

The following biochemical tests are positive: Simmons' citrate, malonate test, lysine and ornithine decarboxylase, tetrathionate reduction, Tween 80 esterase, β-galactosidase, β-xylosidase, and esculin hydrolysis; fermentation of adonitol, L-arabinose, D-dulcitol, D-fructose, D-galactose, D-glucose, D-glycerol, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, melibiose, α-methylglucoside, raffinose, L-rhamnose, D-ribose, salicine, D-sucrose, D-trehalose.

The following biochemical tests are negative: arginine decarboxylase, indole, H_2S, DNase, gelatinase, Voges-Proskauer, phenylalanine, deaminase, urease, fermentation of inulin, D-melezitose, and L-sorbose.

The following compounds can serve as sole carbon source: adonitol, D-alanine, L-alanine, L-arabinose, L-aspartate, citrate, D-dulcitol, *meso*-erythritol, D-fructose, D-galactose, gluconate, D-glucose, L-glutamate, DL-glycerate, D-glycerol, L-histidine, *myo*-inositol, DL-lactate, D-maltose, D-mannitol, D-mannose, L-proline, putrescine, pyruvate, L-rhamnose, D-ribose, salicin, D-sorbitol, succinate, tagatose (upublished data), and D-trehalose.

The following compounds cannot serve as sole carbon source: adipate, β-alanine, 2-aminobenzoate, 4-aminobenzoate, DL-2-aminobutyrate, DL-3-aminobutyrate, DL-4-aminobutyrate, 5-aminovalerate, amylamine, D-arabinose, L-arginine, azelate, benzoate, benzylamine, benzylformate, betaine, 2,3-butanediol, butylamine, butyrate, caprate, *n*-caproate, D-cellobiose, citraconate, L-citrulline, creatine, diphenylamine, dodecane, ethanol, ethanolamine, ethylene glycol, D-fucose, L-fucose (unpublished data), geraniol, glutarate, glycine, glycolate, hexadecane, heptanoate, hippurate, histamine, 4-hydroxybenzoate, DL-3-hydroxybutyrate, inulin, isobutyrate, L-isoleucine, isophthalate, isopropanol, isovalerate, itaconate, 2-ketoglutarate, L-leucine, levulinate, L-lysine, D-mandelate, L-mandelate, mesaconate, methanol, L-methionine, naphthalene, nicotinate, oxalate, pantothenate, pelargonate, phenol, phthalate, pimelate, *n*-propanol, proponiate, propylene glycol, salicylate, sarcosine, sebacate, spermine, suberate, D-sucrose, D-tartrate, L-tartrate, *meso*-tartrate, terephthalate, L-threonine, trigonelline, tryptamine, D-tryptophan, urate, urea, *n*-valerate, and L-valine (data from Gavini et al., 1979).

Occur in fresh water.

The mol% G + C of the DNA is 48.8–52.5 (T_m).

Type strain: ATCC 29844.

Genus IX. **Hafnia** *Møller 1954, 272*[AL]

RIICHI SAKAZAKI

Haf'ni.a. O.L. fem. n. *Hafnia* the old name for Copenhagen.

Straight rods, ~1.0 μm in diameter and 2.0–5.0 μm in length. Conform to the general definition of the family *Enterobacteriaceae*. Not encapsulated. Gram-negative. **Motile by peritrichous flagella at 30°C**, but nonmotile strains may occur. Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Grow readily on ordinary media. Colonies on nutrient agar are generally 2–4 mm in diameter, smooth, moist, translucent, and gray with a shiny surface and entire edge. Oxidase-negative. Catalase-positive. Chemoorganotrophic. **The majority of strains utilize citrate, acetate and malonate as a sole carbon source** after 3–4 days of incubation. Nitrate is reduced to nitrite. H_2S is not produced in the butt of Kligler iron agar. Gelatinase, lipase, and deoxyribonuclease are not produced. Alginate is not utilized. Pectate is not decomposed. Phenylalanine deaminase is not produced. **Lysine and ornithine decarboxylase tests are positive**, but the arginine dihydrolase test is negative. Glucose is fermented with the production of acid and gas. **Acid is not produced from D-sorbitol, raffinose, melibiose, D-adonitol and *myo*-inositol.** The methyl red test is usually positive at 35°C and negative at 22°C. Acetylmethylcarbinol is usually produced from glucose at 22–28°C but may not be produced at 35°C. Occur in the feces of man and other animals including birds; also occur in sewage, soil, water and dairy products. The mol% G + C of the DNA is 48–49 (T_m).

Type species: *Hafnia alvei* Møller 1954, 272.

Further Descriptive Information

Members of *Hafnia* are able to grow at 35°C, but many of their physiological and biochemical activities at this temperature are irregular. Many strains are nonmotile at 35°C, but the majority are motile at 25–30°C. Although most strains do not produce acetylmethylcarbinol from glucose at 35°C, they give a positive Voges-Proskauer reaction when incubated at 22–28°C. At 25°C, they produce gas from glucose and about 10% of them grow on Simmons' citrate agar within 24 h, but all of these reactions may be negative at 35°C.

Lactose is not fermented, but plasmid-mediated lactose-positive strains may occur (Le Minor and Coynault, 1976).

Hafnia is defined as an H_2S-negative organism. Møller (1954) and Kauffmann (1954) reported *Hafnia* as producing H_2S since most strains of *Hafnia alvei* slightly darken ferric chloride-gelatin medium (Kauffmann, 1951) and SIM medium (Difco), as well as peptone iron agar (Difco). They fail, however, to blacken the butt of Kligler iron agar and of triple-sugar iron agar. Ewing (1960) suggested that either Kligler iron agar or triple-sugar iron agar must be a standard medium for the H_2S test of the family *Enterobacteriaceae*, because each permits easy differentiation of genera or species within the family.

The maximum temperature for growth is usually 40–42°C. No growth occurs at 5°C.

The serology of *Hafnia* was first studied by Stuart and Rustigian (1943) who divided their cultures of biotype 32011, the majority of which are now classified into *Hafnia*, into eight serovars. Eveland and Faber (1953) studied 58 strains of biotype 32011 serologically and reported 21 somatic and 22 flagellar antigens. Deacon (1952) also carried out a serological study on 17 cultures of "*Aerobacter cloacae*" including biotype 32011 and recognized 12 somatic and 6 flagellar antigens among the cultures. However, Sakazaki and Namioka (1957) and Sakazaki (1961) found that cultures of biotype 32011 studied by those authors mentioned above included not only *Hafnia* but also *Enterobacter cloacae*. Serological studies on 294 biochemically well-defined *Hafnia* cultures were performed by Sakazaki (1961) who established an antigenic schema of *Hafnia* consisting of 29 O groups and 23 H antigens. Later, Matsumoto (1963, 1964) expanded this schema to 68 O groups and 34 H antigens. Deacon (1952) reported the diaphasic variation in the H antigens of the strains he studied, but Sakazaki (1961) and Matsumoto (1963) failed to observe such variation. Some *Hafnia* strains may be O-inagglutinable with their homologous O antisera in unheated cultures. Sakazaki (1961) suggested that the antigen that inhibited the O-agglutination was a slime antigen. The alpha antigen (Stamp and Stone, 1944) may be recognized in some strains (Sakazaki, 1961; Emslie-Smith, 1961). In addition to this, intergeneric relationships of O antigens were recognized between *H. alvei* and other genera of the family *Enterobacteriaceae* (Sakazaki, 1961; Matsumoto, 1963, 1964; Sedlák and Slajsová, 1966). Eveland and Faber (1953) reported O antigenic relationships between *Hafnia* (biotype 32011) and *Salmonella*.

Baturo and Raginskaya (1978) have recently published an antigenic schema including 39 O and 35 H antigens of *H. alvei*, independent of that of previous investigators.

Table 5.36.
Differential characteristics of the genus **Hafnia** *and biochemically similar genera*[a]

Characteristics	*Hafnia*	*Enterobacter*	*Serratia*
Citrate (Simmons')	−[b]	+	+
Gelatin hydrolysis	−	D	+
Lysine decarboxylase	+	D	D
Arginine dihydrolase	−	D	−
Lipase (Tween 80)	−	−	+
Deoxyribonuclease	−	D	+
Acid from carbohydrates:			
Raffinose, sucrose	−	+	D
Lactose, D-adonitol, *myo*-inositol, D-sorbitol	−	D	D
Hafnia specific bacteriophage lysis[c]	+	−	−
Mol% G + C of DNA	48–49	52–60	52–60

[a] Symbols: +, 90–100% of strains are positive; −, 90–100% of strains are negative; D, different reaction given by different species of a genus.
[b] Late positive reactions are given by ~50% of the strains of *Hafnia*.
[c] Guinée and Valkenburg (1968).

Table 5.37.
Characteristics of **Hafnia alvei**[a]

Characteristics	*H. alvei*
Indole production	−
Voges-Proskauer test (22°C)	+
Voges-Proskauer test (35°C)	d
Citrate (Simmons') (22°C)	d
Citrate (Simmons') (35°C)	−
H_2S (triple-sugar iron agar)	−
Urease (Christensen)	−
Gelatin hydrolysis	−
Phenylalanine deaminase	−
Lysine decarboxylase	+
Arginine dihydrolase	−
Ornithine decarboxylase	+
Growth in KCN medium	+
Malonate utilization	d
Esculin hydrolysis	−
Lipase (Tween 80)	−
Deoxyribonuclease	−
ONPG hydrolysis[b]	d
Gas from glucose	+
Acid from carbohydrates:	
D-Glucose, L-arabinose, maltose, L-rhamnose, trehalose, D-xylose, D-mannitol, glycerol	+
Lactose, melibiose, raffinose, sucrose,[c] D-adonitol, dulcitol, D-sorbitol, *myo*-inositol, mucate	−
Salicin	d
d-Tartrate (Kauffmann-Petersen)	−

[a] For symbols see standard definitions.
[b] ONPG, *o*-nitrophenyl-β-D-galactopyranoside. This test is generally positive especially if it is carried out from a culture incubated at 22°C.
[c] Late positive reactions are given by ~50% of the strains of *Hafnia*.

The majority of strains of *H. alvei* are susceptible to carbenicillin, streptomycin, tetracycline, polymyxin B, and nalidixic acid, but resistant to cephalosporins and ampicillin.

A *Hafnia*-specific bacteriophage that provides a reliable tool for the identification of *Hafnia* strains was described by Guinée and Valkenburg (1968).

H. alvei occurs not only in man and animals and birds, but also in natural environments such as soil, sewage and water. In medical bacteriology, *H. alvei* is found in clinical specimens, especially from feces in healthy humans, occasionally from blood, sputum, urine, and from wounds, abscesses, the throat, abdominal cavity and autopsies. In most cases, however, they are found in mixed culture and seem to be opportunistic pathogens which produce infections in patients with some underlying illness or predisposing factors.

H. alvei has been reported as a possible causative agent of intestinal disorders by some investigators. However, no conclusive evidence has been obtained on its enteropathogenicity. Matsumoto (1963) reported the isolation of this organism from 13% of stool specimens from apparently healthy individuals. Sakazaki (1966, unpublished data) found *H. alvei* in 42% of fecal samples of healthy persons.

Enrichment and Isolation Procedures

Hafnia can grow on less-selective isolation media for enterobacteria such as eosin-methylene blue, deoxycholate-lactose, MacConkey, xylose-lysine-deoxycholate and Hektoen enteric agars. The majority of *Hafnia* strains may also grow on salmonella-shigella and deoxycholate-citrate agars. Colonies of *H. alvei* on these plating agar media are of colorless and translucent and resemble those of *Salmonella* (*Hafnia* strains are sometimes misidentified as *Salmonella* H_2S-negative) but rare strains may produce red or pink colonies on media which contain sucrose. Sakazaki (1966, unpublished data) devised a differential isolation medium, deoxycholate-lactose-sucrose-sorbitol agar.*

There are no selective enrichment broth media for the isolation of *H. alvei*. Some strains fail to grow in selenite and tetrathionate broths.

Maintenance Procedures

Stock cultures may be maintained at room temperature in a semisolid medium consisting of 1.0% Bacto-casitone (Difco), 0.3% yeast extract, 0.5% NaCl and 0.3% agar, pH 7.0. *Hafnia* strains remain viable up to a year without subculture if the culture is sealed with a rubber stopper or a cork which has been soaked in hot paraffin wax. Strains may also be preserved indefinitely by lyophilization.

Differentiation of the genus **Hafnia** *from other genera*

Table 5.36 indicates the characteristics of *Hafnia* that differentiate it from biochemically similar genera.

Taxonomic Comments

The bacteria of the genus *Hafnia* have been described under several names. Møller (1954) found a new group of organisms, in which a supposedly authentic strain of *Bacillus paratyphi-alvei* of Bahr (1919) was included. He proposed the name *Hafnia alvei* for this bacterial group, because he considered that Bahr's strain ought to be regarded as the type of this group. Sakazaki (1961) suggested a new combination *Enterobacter alvei* for *H. alvei*, because of its biochemical similarity to *Enterobacter*. Ewing and Fife (1968) pointed out that Bahr's strain, which had been designated as the type strain of *H. alvei* by Møller (1954), was not an authentic strain of this species, since biochemical reactions of the strain were not the same as those described by Bahr (1919). They considered therefore that the specific epithet *alvei* was

* Deoxycholate-lactose-sucrose-sorbitol agar (per liter of distilled water): yeast extract, 5.0 g; trypticase (BBL), 5.0 g; lactose, 10.0 g; sucrose, 5.0 g; D-sorbitol, 10.0 g; sodium deoxycholate, 2.5 g; sodium citrate, 20.0 g; ferric citrate, 1.0 g; neutral red, 0.02 g; agar, 15.0 g. The medium is adjusted to pH 7.4.

illegitimate, and proposed the name *Enterobacter hafniae* for *H. alvei*. However, *Hafnia alvei* Møller 1954 is the only correct name for this group of bacteria, because there is no doubt that the Bahr's strain studied by Møller (1954) was a new bacterium at that time. In addition, numerical taxonomy studies by Johnson et al. (1975) and Gavini et al. (1976) indicated that *Hafnia* strains occupy a position separate from *Enterobacter*. In DNA/DNA hybridization studies, Steigerwalt et al. (1976) reported only 11–26% homology between *H. alvei* and *Enterobacter*.

Only a single species, *Hafnia alvei*, has been designated. Steigerwalt et al. (1976) indicated that *H. alvei* consists of two DNA relatedness groups, but these two groups have not been defined biochemically.

Priest et al. (1973) proposed that *Obesumbacterium proteus* Shimwell 1964, a common brewery contaminant, should be placed in the genus *Hafnia* as *H. protea*. They described two groups in this species by numerical analysis of biochemical and physiological characteristics. Brenner (1979, personal communication) determined DNA relatedness in both groups and found that one group appears to be a biovar of *H. alvei*, whereas the other group is a new species that does not belong to the genus *Hafnia* (see article on "Other Genera of the Family *Enterobacteriaceae*").

List of the species of the genus Hafnia

1. **Hafnia alvei** Møller 1954, 272.[AL]

al've.i. L. n. *alveus* a beehive; L. gen. n. *alvei* of a beehive.

The morphology is as given for the genus. Motility is most pronounced at 30°C and often absent at 37°C. Nonmotile strains may be encountered occasionally. Capsules are usually not present.

Grows readily on ordinary media. Colonies are translucent. Rare strains may produce mucoid colonies. The majority of strains grow on salmonella-shigella agar.

Physiological and biochemical characteristics are presented in Tables 5.36 and 5.37.

Found in the feces of man and other animals, including birds. Also found in sewage, soil, water and daily products.

The mol% G + C of the DNA is 48.0–48.7 (T_m).

Type strain: NCTC 8106 (ATCC 13337).

Genus X. **Edwardsiella** *Ewing and McWhorter 1965, 37*[AL]

JOHN J. FARMER III AND ALMA C. MCWHORTER

("Asakusa group" Sakazaki and Murata 1962, 616; "Bartholomew group" King and Adler 1964, 230; "Bacterium 1483-59" Ewing et al., 1965, 33.)

Ed.ward.si.el'la. M.L. dim. ending *ella*; M.L. fem. n. *Edwardsiella*; named after P. R. Edwards (1901–1966), the American bacteriologist who was chief of the Enteric Laboratories, Centers for Disease Control, U.S.A., from 1948–1962 and made many contributions to our knowledge of the *Enterobacteriaceae* (Cherry and Ewing, 1966).

Small straight rods, about 1 μm in diameter × 2–3 μm conforming to the general definition of the family *Enterobacteriaceae*. Gram-negative. **Motile** by peritrichous flagella. Facultatively anaerobic. Catalase-positive. Oxidase-negative. Reduce nitrate to nitrite. Optimum temperature, 37°C, except for *E. ictaluri* which prefers a lower temperature. Growth occurs on peptone and similar agar media with small colonies (~0.5–1 mm in diameter) after 24 h incubation. **Vitamins and amino acids are required for growth.** Ferment D-glucose with the production of acid and often visible gas. Also ferment a few other compounds **but are inactive compared to many taxa in the family *Enterobacteriaceae*. Usually resistant to colistin but have large zones around most other antibiotic disks, including penicillin.** Frequently isolated from cold-blooded animals and their environment, particularly fresh water. Pathogenic for eels, catfish, and other animals, sometimes causing economic losses; also a rare opportunistic pathogen for humans. The mol% G + C of the DNA is 53–59 (T_m, Bd).

Type species: *Edwardsiella tarda* Ewing and McWhorter 1965, 37.

Further Descriptive Information

Most of the available information concerns *E. tarda* since the other two species of *Edwardsiella* have been described only recently. *Edwardsiella* strains grow less luxuriantly than many other *Enterobacteriaceae* and form smaller colonies in 24 h at 36°C. This may be related to their growth requirements. d'Émpaire (1969) reported that *E. tarda* requires cysteine, methionine and nicotinamide. Hoshina (1962) stated that "*Paracolobactrum anguillimortiferum*," an organism now thought to have been a *Edwardsiella (E. anguillimortifera)*, required niacin, phenylalanine, threonine and valine, and that aspartic acid, glutamic acid, isoleucine and cysteine were "important for growth." Other groups of *Edwardsiella* probably have similar nutritional requirements.

E. ictaluri is the most fastidious species of the genus. Growth is very slow on plating media and 2–3 days of incubation are often required for colonies to reach 1 mm in diameter. Although characteristic biochemical reactions are apparent at 36°C (see Tables 5.38 and 5.39), a lower temperature seems to be preferred (Hawke, 1979). Biochemically, *E. ictaluri* is also the least active of the *Edwardsiella* species.

Two independent serotyping schemes have been described for *E. tarda*. Sakazaki (1967) recognized 17 O antigens, 11 H antigens, and 18 O-H combinations. Edwards and Ewing (1972) described a scheme with 49 O antigens, 37 H antigens, and 148 O-H combinations among 394 cultures studied. Currently, efforts are being made to standardize the schema for serotyping *Edwardsiella* (R. Sakazaki and D. J. Brenner, personal communication). This schema will be a combination of the O and H antigens described by Ewing and McWhorter (Edwards and Ewing, 1972, p. 145) and those of Sakazaki (1967). Other typing techniques such as bacteriocin production or susceptibility, bacteriophage typing, and biotyping have seldom been used for *Edwardsiella*, although Hamon et al. (1969) did demonstrate bacteriocin production and sensitivity.

Many strains of *Edwardsiella* have high-level intrinsic resistance to colistin (Muyembe et al. 1973), but some strains have small zones of inhibition around colistin-impregnated disks. All three species have large zones around penicillin-impregnated disks, an unusual finding for members of *Enterobacteriaceae*. They also have large zones of inhibition around most other antibiotics. Occasionally resistance to sulfonamides or other drugs has been observed in *E. tarda*. Antibiotic resistance that is mediated by R plasmids (R factors) is very rare in *Edwardsiella* (no examples were encountered in our survey of the literature), which suggests that human contact with this genus of organisms is rare.

When *E. tarda* was first described, it was thought to be a possible cause of diarrhea (Ewing et al., 1965). Some intriguing evidence later came from a study of the Orang Asli, a group of jungle-dwelling natives of West Malaysia (Gilman et al., 1971). There were 29 isolates of *E. tarda* from stool cultures of 208 patients hospitalized with blood diarrhea but only one isolate from 120 stool cultures of control individuals (hospital patients without diarrhea). An interesting relationship between *E. tarda* and the protozoan *Entamoeba histolytica* was also shown. Twenty-five of the patients with bloody diarrhea had both organisms, and 4 had *E. tarda* only. Twenty-four of the 25 patients with both organisms had significant antibody titers to a whole-cell antigen of *E. tarda*, whereas a control group of 15 patients was negative. All of the

patients who were culture-negative for *E. tarda* but positive for *Entamoeba histolytica* also had antibodies to *E. tarda*. These data indicate that *E. tarda* may be involved in the pathogenesis of amoebic dysentery, although an alternate explanation is that the presence of *E. tarda* is due to a change in the gut micro-environment and that the organism plays no role in diarrhea. Makulu et al. (1973) also found an association between *E. tarda* and *Entamoeba histolytica* in patients from Zaire with bloody diarrhea, but the correlation was lower than in the previous study. They postulated a possible triggering role of *E. tarda* in initiating invasive amoebic infection.

E. tarda is rarely present in the feces of healthy people. Onogawa et al. (1976) in Japan found only one positive culture from 97,704 food handlers and only 25 positive cultures from 255,896 school children. Makulu et al. (1973) found no positive cultures among 841 healthy subjects in Zaire. Several studies indicate that the number of *E. tarda* isolations depends upon the methods used in processing stool cultures, the geographic area of the study, and the season in which the survey is done (Iveson, 1973). These variables have not always been considered by those trying to determine the relative incidence of *E. tarda* in patients with diarrhea and in controls. A higher isolation rate has invariably been found among the diarrhea patients (Bhat et al., 1967; Ewing et al., 1965; Gilman et al., 1971; Makulu et al., 1973; Nguyen-Van-Ai et al., 1975). Some strains of *E. tarda* may be able to cause diarrhea, particularly in underdeveloped countries, but *E. tarda* should not be considered as an "inherent pathogen," a status given to *Salmonella* and *Shigella*. The role of *Edwardsiella* in diarrhea needs further study. One promising technique is to test a patient's acute-phase and convalescent-phase sera against the particular strain of *Edwardsiella* isolated from feces. Chatty and Gavan (1968) reported a case in which *E. tarda* was isolated from a patient with nutritional cirrhosis of the liver, diarrhea, and low-grade fever. The person had lived in Central and South America. A convalescent-phase serum from the patient had an antibody titer of 1:160 to both somatic and flagella antigens of the *E. tarda* strain isolated from feces. In this case *E. tarda* was incriminated as the probable cause of the diarrhea. Similar studies are needed for all isolates of *Edwardsiella* from stools of people with diarrhea and from healthy controls.

E. tarda is now well documented as an opportunistic pathogen, but it is rarely found in most industrialized countries. It seldom causes meningitis, endocarditis, bacteremia, or urinary tract infections but is often isolated from wounds (Jordan and Hadley, 1969). A typical example is the report of Chatty and Gavan (1968) of a boy who struck a submerged log while swimming in a lake. A splinter entered his right thigh and eventually led to gas gangrene, a diagnosis confirmed by the isolation of *Clostridium perfringens*. *E. tarda* was also isolated but probably only colonized the wound. Wound cultures have often yielded other bacteria in addition to *E. tarda*, so its role is difficult to assess. Antibody responses to the particular strain would be very useful in defining the role of *E. tarda* in these infections.

E. tarda has been isolated from many animals including pets (Nguyen-Van-Ai et al., 1975), domestic animals (Owens et al., 1974), animals in zoos (Otis and Behler, 1973), rats (Nguyen-Van-Ai et al., 1975), aquatic animals and birds (White et al., 1973), fish (Nguyen-Van-Ai et al., 1975), frogs (Bartlett et al., 1977), turtles (Otis and Behler, 1973), and marine animals (Nguyen-Van-Ai et al., 1975). It is also frequently found in the environment, particularly where these animals live (White et al., 1973). Most *E. tarda* isolates have come from stools or other specimens from healthy animals, but *E. tarda* can cause outbreaks of "red disease" in pond-cultured eels (Wakabayashi and Egusa, 1973) or of "emphysematous putrefactive disease" (gas-filled lesions in the muscles) of channel catfish (Meyer and Bullock, 1973). Isolated cases of septicemia have been reported in other animals (Chamoiseau, 1967).

Another *Edwardsiella* species, *E. hoshinae* is also associated with animals, but only eight isolates were originally reported (Grimont et al, 1980). Three were from monitor lizards (*Varanus* sp.) in Chad, two from puffins (*Fraterciula arctica*) in Brittany, France, one from a lizard in Senegal, one from a flamingo (*Phoenicopterus ruber*) in France and one from water. Two recent isolates were from feces of patients without diarrhea (R. Sakazaki, personal communication); thus there is no evidence that *E. hoshinae* can cause human disease. Another distinct group of *Edwardsiella* strains originally called *Edwardsiella* group "GA 7752" (Hawke, 1979) has caused many outbreaks of enteric septicemia of catfish. This new *Edwardsiella*, which was recently named *E. ictaluri* (Hawke et al., 1981), has been isolated from pond-raised catfish in the southeast, particularly the Mississippi delta area where catfish farming is most intense. The disease is seasonal, occurring almost exclusively in the spring and again in the fall when water temperatures are about 25°C, which seems to be the optimum growth temperature of *E. ictaluri* in the laboratory (Hawke, 1979). *E. ictaluri* has also been isolated from white catfish (*Ictalurus catus*) and the brown bullhead (*Ictalurus nebulosus*). No human isolates have been reported.

The natural resorvoir of *Edwardsiella* appears to be the intestine of animals, from which feces disseminate the organism into the environment. Most human infections caused by *E. tarda* probably result from contact with the organism in the environment. Endogenous human infections, although probably rare, may occur if gut carriage has been established.

Enrichment and Isolation Procedures

Very little information is available on the selective isolation of *Edwardsiella*, and most of what exists concerns *E. tarda*, the most common species. Little has been written about the other species because they were described only recently.

Most data on the isolation of *E. tarda* have come from culture surveys to detect *Salmonella* and *Shigella*. Unfortunately, there has been no systematic study to evaluate growth and survival of *E. tarda* in enrichments and on plating media commonly used in enteric bacteriology. *E. tarda* strains usually grow on plating media commonly used, including the following agar media: sheep blood, chocolate, MacConkey, SS (salmonella-shigella) and deoxycholate citrate. However, strains of *E. tarda* do grow more slowly than most other species of *Enterobacteriaceae*. Pure cultures grow on brilliant green and bismuth sulfite agar (Sakazaki, 1967), but Iveson (1973) found these two media useless in isolating *E. tarda* from feces.

Strains of *E. tarda* are often isolated from liquid enrichments such as tetrathionate and selenite F (media used to isolate *Salmonella*) and occasionally these enrichments have resulted in a higher yield than direct plating (Makulu et al., 1973). Iveson (1973) described an efficient method for isolating *E. tarda* from stool cultures. Specimens were first enriched (either at 37°C or 43°C) with strontium chloride B medium* (Iveson, 1971). After 24 h of incubation, plates of deoxycholate citrate agar were streaked. This method was excellent for isolating *Samonella*, *Arizona*, *Shigella*, and *E. tarda* from stool cultures and could presumably be adapted to all types of specimens including those from the environment.

E. tarda and often other *Edwardsiella* species (Farmer and McWhorter, unpublished data) have intrinsic resistance to the polypeptide antibiotic colistin, and advantage can be taken of this for isolation procedures. Muyembe et al. (1973) showed that all *E. tarda* strains grew in the presence of 10 μg/ml of colistin and that more than 80% grew in 100 μg/ml. Other metabolic properties of *E. tarda* could be used in designing a differential and selective medium. Colistin could be added to peptone iron agar so that strains that grow and produce black colonies (because of H_2S production) would probably be *E. tarda* or H_2S-positive species of *Proteus*. A different approach would be to incorporate several carbohydrates (or related compounds) not fermented by *E. tarda* into a fermentation base such as MacConkey agar base without lactose (Difco), but with added colistin. Most *Enterobacteriaceae* would either be inhibited (only *Serratia*, *Proteus*, *Providencia*, *Morganella*, *Cedecea* and some *Yersinia* strains are colistin-resistant)

* Strontium chloride B medium (g/liter): Bacto-tryptone (Difco), 5.0; NaCl, 8.0; KH_2PO_4, 1.0; and $SrCl_2$, 34.0. "Sterilization" is done by heating at 100°C for 30 min (final pH, 5.0–5.5).

or form red colonies because they ferment one or more of the sugars. Colonies of *E. tarda* would be colorless. Various combinations of the carbohydrates used to differentiate the four *Edwardsiella* groups (Table 5.38) could be used to make a differential medium for one of the groups. Many other approaches are feasible which would combine colistin enrichment with a differential biochemical reaction. None of the above methods has actally been tried and only represent theoretical possibilities.

Maintenance Procedures

Edwardsiella strains survive well in the laboratory without transfer. Cultures are inoculated into 100 × 13 mm tubes containing a "peptone" medium (Trypticase soy agar slants, Trypticase soy semisolid (0.4% agar), or blood agar base slants with 0.3% added yeast extract) and are incubated overnight. The tubes are then tightly sealed with a stopper (white rubber, No. 000, for a 13 × 100 mm screw cap tube) or with paraffin-coated corks. It is essential that the seal be airtight so that the water in the medium does not evaporate because drying may kill the strain. Almost all cultures of *E. tarda* have remained viable for over 10 years without transfer with this storage method, but there has been little experience with the other *Edwardsiella* species.

In addition to those "working stocks," important cultures should also be preserved as "freezer stocks." Growth from a Trypticase soy agar plate is removed with cotton swab and a heavy suspension is made in sterile 10% w/w skim milk in water (or in sterile sheep or rabbit blood). This suspension is quick frozen in 95% alcohol (which is kept in −70°C freezer) or in a dry ice-acetone bath. Other workers prefer to put the skim milk suspension into the freezer directly so it is frozen slowly rather than quickly. The freezer stocks should be kept in the freezer at the lowest temperature available. Freeze-drying presumably can also be used for long term preservation.

Procedures for Special Testing

Indole production, method 1. This is the method described by Edwards and Ewing (1972) and is the "standard method" used by the Enteric Section for testing all cultures. A tube of peptone water (20 g of Bacto-peptone (Difco), 5 g of NaCl and 1000 ml of distilled water) is inoculated and incubated at 36°C for 48 h. About 0.6 ml of Kovacs' reagent (10 g of *p*-dimethylaminobenzaldehye, 50 ml of 12 N HCl, 150 ml of isoamyl alcohol) is then added. A positive test is the presence of a pink or red color in the upper layer.

Indole production, method 2. This is a more sensitive method which detects indole production by some strains which are indole-negative by method 1. Heart infusion broth (Difco) is inoculated, incubated for 48 h, and then tested with Kovacs' reagent as described above.

Differentiation of the genus **Edwardsiella** *from other genera*

There is no single test to differentiate *Edwardsiella*. The best method is to do a complete set of biochemical reactions. This will indicate that the culture is a member of *Enterobacteriaceae* and that it belongs to the genus *Edwardsiella*. *Edwardsiella* is more fastidious than many other *Enterobacteriaceae* and forms colonies in 24 h at 36°C which are smaller than those of most other *Enterobacteriaceae*. *Edwardsiella* is apparently more susceptible to 2,4-diamino-6,7-diisopropyl pteridine (vibriostatic compound "O/129", Sigma Chemical) than other *Enterobacteriaceae* (Chatelain et al., 1979, Grimont et al., 1980). Many groups of *Enterobacteriaceae* have zones of inhibition around disks impregnated with the antibiotic colistin but have no zone around penicillin. *Edwardsiella* strains usually have the opposite pattern. *Edwardsiella* is biochemically somewhat similar to *Escherichia coli*, the *Salmonella-Arizona* group and the *Proteus-Providencia-Morganella* group but is easily differentiated on the basis of a complete set of biochemical test results or on the basis of antibiotic susceptibility patterns. These phenotypic differences correlate with the phylogenetic divergence of *Edwardsiella* from these other groups.

Taxonomic Comments

Edwardsiella was discovered independently in 1959 by two research groups. It was called "Bacterium 1483-59" by Ewing and his coworkers at the Centers for Disease Control (Ewing et al. 1965). Almost all of their isolates were from human clinical specimens and most were from feces. In 1959 Sakazaki and coworkers (Sakazaki, 1967) independently discovered the same group of organisms isolated mainly from snakes. The name "Asakusa group" was coined by the Japanese workers (Sakazaki and Murata, 1962). King and Adler (1964) proposed the name "Bartholomew group" in 1964, but it was Ewing and colleagues who in 1965 coined the scientific name *Edwardsiella tarda*, which has standing in nomenclature.

When *Edwardsiella* was proposed, there was some doubt whether it deserved status as a separate genus, since there was only one species. It was even questioned whether *E. tarda* was a separate species. Cowan (and Steel, 1974, p. 105) makes the following statement: "*Edwardsiella* has much in common with some shigellae which, within themselves have differences comparable with those between *Escherichia* and *Edwardsiella*. In short, *Edwardsiella* is a good example of the excessive splitting at 'generic' level that has taken place within the enterobacteria. In our opinion it is better regarded as a biotype of *Escherichia coli*; less satisfactorily as a species, *Escherichia tarda*." However, Brenner and coworkers (1974) showed that 20 strains of *E. tarda* from diverse sources and different countries were highly related by DNA/DNA hybridization (82–96% related at 60°C with small values for percent divergence, 81–93% related at 75°C; done by the hydroxyapatite method with ^{32}P). *Edwardsiella tarda* was only 8–29% related to other genera in the family *Enterobacteriaceae*, and was 17–25% related to *Escherichia coli*, the type species of the type genus for the family. These data argue convincingly that *Edwardsiella* should be maintained as a separate genus in the family *Enterobacteriaceae*. All of the *Edwardsiella* strains studied by Brenner et al. (1974) were highly related to each other and formed a single species. However, two new *Edwardsiella* species have been described in the last two years. *E. hoshinae* is distinct from *E. tarda* by DNA/DNA hybridization (S1 nuclease method; Grimont et al., 1980) and is phenotypically distinct from *E. tarda* and *E. ictaluri*. *E. ictaluri* is distinct from, but closely related to, *E. tarda* by DNA/DNA hybridization (hydroxyapatite method with ^{32}P; Hawke et al., 1981). These additional species now make *Edwardsiella* a much better "phylogenetic genus" and nicely counter the previous argument that there had been excessive splitting in establishing *Edwardsiella*.

A nomenclatural problem in *Edwardsiella* concerns the name *Edwardsiella anguillimortifera* (Hoshina 1962, Sakazaki and Tamura 1975). This name appears on the *Approved Lists of Bacterial Names* (Skerman et al., 1980, p. 292) with the type strain listed as ATCC 15947. This strain was proposed as the neotype strain (Sakazaki and Tamura, 1975) but has been challenged (J. J. Farmer III, 1976–1977, unpublished letters to P.H.A. Sneath, Chariman of the Judicial Commission) under rule 18e of the 1975 Bacteriological Code because it was considered a doubtful name (*nomen dubium*) and because the properties of the proposed neotype strain differed from those given in the original description of "*Paracolobactrum anguillimortiferum*" Hoshina 1962 (see Table 5.40). Furthermore, no other strains have been isolated which fit the original description of "*P. anguillimortiferum*." It could be argued that ATCC 15947 became established as the type strain of "*P. anguillimortiferum*" with the implementation of the *Approved Lists of Bacterial Names* (Skerman et al., 1980, page 229, paragraph 4). An extension of this argument might be that since "*anguillimortifera*" is the senior synonym, it must replace "*tarda*." Although there is possible nomenclatural validity to this argument, we believe that *E. tarda* is the name that should be used to avoid unnecessary confusion in the literature and provide stability in nomenclature. The controversy is quite complex (involving two different versions of the *Bacteriological*

Code, as well as the *Approved Lists of Bacterial Names*), and will eventually require a ruling of the Judicial Commission. In the meantime, we consider "*Paracolobactrum anguillimortiferum*" as only a possible subjective synonym of *Edwardsiella tarda* and consider the *E. anguillimortifera* as being under judicial consideration (*sub judice*) and will not use it. Since its proposal in 1975, few workers have used *E. anguillimortifera* but instead have used the well known and accepted name *E. tarda*. We will follow this convention.

Acknowledgments

We thank W. H. Ewing for helpful discussions and for his recollections of the early days of *Edwardsiella* research, Riichi Sakazaki for interesting discussion about his experience with *Edwardsiella* and about the problem of "*Paracolobactrum anguillimortiferum*," P. A. D. Grimont who furnished strains of *E. hoshinae* and *E. tarda* biogroup 1, J. P. Hawke who furnished strains of *Edwardsiella ictaluri*, and Kathleen Ann Kelley of Emory University for assistance in writing the Greek and Latin origins of scientific names.

Further Reading

Ewing, W. H., A. C. McWhorter, M. R. Escobar, and A. H. Lubin. 1965. *Edwardsiella*, a new genus of *Enterobacteriaceae* based on a new species, *E. tarda*. Int. Bull. Bacteriol. Nomen. Taxon. *15:* 33–38.

Farmer, J. J. III. 1981. The genus *Edwardsiella*. In Starr, Stolp, Trüper, Balows and Schlegel (Editors), *The Prokaryotes*. Springer-Verlag, New York, pp. 1135–1139.

Grimont, P. A. D., F. Grimont, C. Richard and R. Sakazaki. 1980. *Edwardsiella hoshinae*, a new species of *Enterobacteriaceae*. Curr. Microbiol. *4:* 347–351.

Hawke, J. P. 1979. A bacterium associated with disease of pond cultured channel catfish, *Ictalurus punctatus*. J. Fisheries Res. Board Canada *36:* 1508–1512.

Hawke, J. P., A. C. McWhorter, A. G. Steigerwalt, and D. J. Brenner. 1981. *Edwardsiella ictaluri* sp. nov., the causative agent of enteric septicemia of catfish. Int. J. Syst. Bacteriol. *31:* 396–400.

Jordon, G. W. and W. K. Hadley. 1969. Human infection with *Edwardsiella tarda*. Ann. Intern. Med. *70:* 283–288

Differentiation and characteristics of the species and biogroups of the genus Edwardsiella

Table 5.38 presents the differential characteristics of the species and biogroups of *Edwardsiella*. Table 5.40 presents additional biochemical features of the organisms.

List of the species of the genus Edwardsiella

1. **Edwardsiella tarda** Ewing and McWhorter 1965, 37[AL] in Ewing, McWhorter, Escobar and Lubin (1965).

tar'da. L. fem. adj. *tarda* slow (intended meaning was "inactive," referring to the fermentation on only a few carbohydrates compared to many other *Enterobacteriaceae*).

The characteristics are as given for the genus and listed in Tables 5.38 and 5.39. Grimont et al. (1980) recently described a group of D-mannitol-positive, sucrose-positive, L-arabinose-positive strains which were closely related to "biochemically" typical strains of *E. tarda* by DNA/DNA hybridization. In this chapter this phenotypically distinct group will be referred to as "*E. tarda* biogroup 1" (see Tables 5.38 and 5.39). Strains of *E. tarda* which are negative for D-mannitol, sucrose and L-arabinose are much more common (perhaps a thousand times so), and are designated as "*E. tarda* wild type."

As indicated previously under Taxonomic Comments, the name *Edwardsiella anguillimortifera* (Hoshina 1962) Sakazaki and Tamura 1975, might be considered to be an objective synonym of *E. tarda* because it has the same type strain (ATCC 15947) on the *Approved Lists of Bacterial Names* (Skerman et al., 1980). The status of this strain, however, has been challenged because the characteristics of the strain differ in several important respects from Hoshina's description of "*Paracolobactrum anguillimortiferum*" (see Table 5.40), and the matter will need clarification by the Judicial Commission.

Occurs in a wide variety of animals, rarely in the feces of healthy people. It is an opportunistic human pathogen, which may cause wound infections and probably also some cases of diarrhea.

The mol% G + C of the DNA of *E. tarda* is 55–58 (T_m, Bd).

Type strain (holotype): ATCC 15947.

2. **Edwardsiella hoshinae** Grimont, Grimont, Richard and Sakazaki, 1981, 216.[VP] (Effective publication: Grimont, Grimont, Richard and Sakazaki, 1980, 349.)

ho.shi'nae. M.L. gen. n. *hoshinae* of Hoshina; named after the late Toshikazu Hoshina, the Japanese bacteriologist who was one of the first to decribe an organism which was probably an *Edwardsiella*.

The characteristics are as described for the genus and indicated in Tables 5.38 and 5.39.

Most isolates have come from animals. However, two human isolates were from human feces but there is no evidence that this species causes diarrhea.

Four of the available isolates of *E. hoshinae* have no zones of inhibition around disks impregnated with the antibiotic colistin; however, the four other strains have zones of 10–17 mm. *E. hoshinae* has large zone around the following antibiotics: nalidixic acid, sulfadiazine, gentamicin, streptomycin, kanamycin, tetracycline, chloramphenicol, ampicillin, carbinicillin, cephalothin and penicillin G (range of zones of inhibition for penicilin G, 28–31 mm).

The mol% G + C of the DNA is 56–57 (T_m).

Type strain: (holotype): ATCC 33379 (CIP 78-56, Grimont 2-78).

3. **Edwardsiella ictaluri** Hawke, McWhorter, Steigerwalt and Brenner 1981, 400.[VP] (*Edwardsiella* GA 7752 Hawke, 1979, 1509.)

ic.ta.lu'ri. *Ictalurus* the genus name for catfish; M.L. fem. adj. *ictaluri* pertaining to catfish.

The chacteristics are as described for the genus and as indicated in Tables 5.38 and 5.39.

E. ictaluri is the most fastidious of the three *Edwardsiella* species. Growth is very slow on plating media, often requiring 2 or 3 days of incubation for colonies to become 1 mm in diameter. It seems to prefer a lower temperature, although characteristic biochemical reactions are apparent at 36°C (Tables 5.38 and 5.39). Biochemically, it is also the least active of the three *Edwardsiella* species.

Table 5.38.
Differentiation of the species and biogroups of the genus **Edwardsiella**[a]

Characteristics	1. *E. tarda* Wild Type	1. *E. tarda* Biogroup 1	2. *E. hoshinae*	3. *E. ictaluri*
Acid production from:				
D-Mannitol	−	+	+	−
Sucrose	−	+	+	−
Trehalose	−	−	+	−
L-Arabinose	−	+	[−]	−
Tetrathionate reduction[b]	+	−	+	
Malonate utilization	−	−	+	−
Indole production (method 1)	+	+	[−]	−
H_2S production on triple-sugar iron agar	+	−	−	−
Motility	+	+	+	−
Citrate (Christensen's)	+	+	[+]	−

[a] Symbols: +, positive for 90–100% of strains (at 36°C in 48 h); [+], positive for 75–89% of strains; [−], positive for 11–25% of strains; −, positive for 0–10% of strains.

[b] Based on the data of Grimont et al. (1981).

Table 5.39.
Other characteristics of the species and biogroups of the genus **Edwardsiella**[a]

Characteristics	1. *E. tarda* Wild Type	1. *E. tarda* Biogroup 1	2. *E. hoshinae*	3. *E. ictaluri*
Indole production[b]:				
Method 1	+	+	[−]	−
Method 2	+	+	d[w]	
Methyl red	+	+	+	−
Voges-Proskauer	−	−	−	−
Citrate:				
Simmons'	−	−	−	−
Christensen's	+	+	[+]	−
H_2S production:				
Triple-sugar iron agar	+	−	−	−
Peptone iron agar	+	[+][w]	+[w]	−
Urea (Christensen's)	−	−	−	−
Phenylalanine deaminase	−	−	−	−
Amino acid decarboxylases (Møller's)				
Lysine decarboxylase	+	+	+	+
Arginine dihydrolase	−	−	−	−
Ornithine decarboxylase	+	+	+	+[w]
Motility:				
36°C	+	+	+	−
25°C (within 3 d)	+	d	+	+[w]
Gelatin hydrolysis (22°C)	−	−	−	−
KCN, growth in	−	−	−	−
Malonate utilization	−	−	+	−
Gas production, D-glucose	+	−	[−]	d
Gas production, any sugar	+	+	d	+
Acid production from:				
D-Mannose, maltose	+	+	+	+
D-Mannitol, sucrose	−	+	+	−
Trehalose	−	−	+	−
L-Arabinose	−	+	[−]	−
Glycerol	d	−	d	−
Salicin	−	−	d	−
Adonitol, D-arabitol, cellobiose, dulcitol, erythritol, lactose, *i*-(*myo*)-inositol, melibiose, α-methyl-D-glucoside, raffinose, L-rhamnose, D-xylose	−	−	−	−
Acid production from mucate	−	−	−	−
Tartrate (Jordan's)	[−]	−	−	−
Esculin hydrolysis	−	−	−	−
Acetate utilization	−	−	−	−
Nitrate reduced to nitrite	+	+	+	+
Deoxyribonuclease	−	−	−	−
Lipase (corn oil)	−	−	−	−
β-Galactosidase (ONPG[c] test)	−	−	−	−
Pectate hydrolysis	−	−	−	−
Pigment production	−	−	−	−
Tyrosine clearing	−	−	−	−
Oxidase test (Kovacs')	−	−	−	−
Tetrathionate reductase[d]	+	−	+	
Mol% G + C of DNA	55–58		56–57	53

Symbols: +, positive for 90–100% of strains (at 36°C in 48 h unless otherwise indicated); [+], positive for 75–89% of strains; [−], positive for 11–25% of strains; −, positive for 0–10% of strains; d, positive for 26 to 74% of strains; superscript "w", weak reaction.
Grimont et al. (1981) reported that all eight strains of *E. hoshinae* were indole-positive. In our hands two strains were indole-negative and the others produced small amounts.
ONPG, *o*-nitrophenyl-β-D-galactopyranoside.
Based on the data of Grimont et al. (1981).

Table 5.40.
Differences in the reported phenotypic properties of **Edwardsiella tarda** *and* **"Paracolobactrum anguillimortiferum"**[a]

Property[b]	*E. tarda*	*"P. anguillimortiferum"*
Methyl red test	+	−
Phenylalanine required	−	+
Threonine required	−	+
Valine required	−	+
Cysteine required	+	−
Methionine required	+	−
Pathogenic for trout[c]	−	+

[a] Symbols: see standard definitions.
[b] Results for the methyl red test for *E. tarda* are based on data obtained in the Enteric Section. The nutritional requirements of *E. tarda* are as given by d'Empaire (1969). Properties of *"P. anguillimortiferum"* are based on the original description by Hoshina (1962). Two different workers (unpublished results) have not been able to duplicate the work of d'Empaire on the nutritional requirements of *E. tarda*, so this point needs clarification. The properties of *"P. anguillimortiferum"* cannot be verified because no authentic strains are available.
[c] Meyer and Bullock (1973) found no pathogenicity for "fingerling brown trout" (*Salmo trutta*); Hoshina (1962) found pathogenicity for rainbow trout.

The antibiotic susceptibility by disk diffusion is difficult to determine because the strains grow so poorly on Mueller-Hinton agar at 37°C. They must be incubated at 25°C instead. If 12 antibiotic disks are placed on a plate, it cannot be read after incubation because the large zones of inhibition overlap and no growth is visible. Instead, it is preferable to place only four disks on each of three 150 × 20 mm Mueller-Hinton agar plates. After 24 h at 25°C the growth is too faint to read, but at 48 h zones of inhibition are clear. No zone of inhibition occurs around colistin (10 μg disk) but very large (20–50 mm) zones were around the other agents tested, including a large zone (range, 29–35 mm) around penicillin G (10 U/disk). Thus, *E. ictaluri* appears to be susceptible to nalidixic acid, sulfadiazine, streptomycin, kanamycin, gentamicin, tetracycline, chloramphenicol, penicillin, ampicillin, carbenicillin and cephalothin but resistant to colistin.

Occurs as a pathogen of catfish.

The mol% G + C of the DNA is 53 (Bd).

Type strain (holotype): ATCC 33202 (CDC 1976-78, GA 7752).

Genus XI. **Proteus** *Hauser 1885, 12.*[AL]

JOHN L. PENNER

Pro'te.us. Gr. n. *Proteus*: an ocean god able to change himself into different shapes.

Straight rods, 0.4–0.8 μm in diameter × 1.0–3.0 μm in length. Gram-negative. **Motile** by peritrichous flagella. **Most strains swarm with periodic cycles of migration producing concentric zones, or spread in a uniform film, over moist surfaces** of nutrient media solidified with agar or gelatin. The organisms in this genus conform to the definition of the family *Enterobacteriaceae*. They **oxidatively deaminate phenylalanine and tryptophan. Urea is hydrolyzed.** They produce acid from several mono- and disaccharides. They do not produce acid from inositol or from straight chain tetra-, penta- or hexahydroxy alcohols, but generally do produce acid from glycerol. **Hydrogen sulfide is produced.** Pathogenic, causing urinary tract infections; also are secondary invaders, causing septic lesions at other sites of the body. Occur in the intestines of humans and a wide variety of animals; also occur in manure, soil and polluted waters. One species has been isolated only from gypsy moth larvae. The mol% G + C of the DNA is 38–41 (T_m) (Falkow et al., 1962).

Type species: *Proteus vulgaris* Hauser 1885, 12.

Further Descriptive Information

In broth cultures, the cells are short rods about 0.6 μm wide and 1.2 μm long. On solid media, cells are 0.8 μm wide and 1.2 μm long (Williams, 1978). Swarming (the movement of cells in periodic cycles of migration and consolidation) occurs on media solidified with agar or gelatin to produce concentric rings on the plate around the point of inoculation. During migration, the cells (swarm cells) are 20–80 μm long and possess many flagella. During consolidation, swarm cells divide for a period of time before producing another generation of swarm cells (Williams, 1978). Some strains (or variants) produce a single uniform film without periodic cycles (C variant of Belyavin, 1951, and the Z variant of Coetzee and Sacks, 1960). Some strains neither swarm nor spread and merely form distinct colonies.

New insights into the phenomenon of swarming have been gained in the last decade but the mechanisms basic to the induction of swarming remain a mystery. Factors critical to the initiation of swarming appear to be the development of the elongated swarm cells, the increased manufacture of flagella and the production of extracellular slime.

The swarming of *Proteus* makes it difficult to isolate bacteria of other species from pathological specimens plated on agar media and therefore methods have been contrived to prevent swarming. In the enteric laboratory, media have been formulated to inhibit swarming by incorporating in the media bile salts or detergents, by reducing the sodium chloride concentration or by increasing the concentration of the agar to 4% (New Zealand agar) or 7% (Japanese agar). The incorporation of 0.1–0.3 mM *p*-nitrophenyl glycerol in solid media also inhibits swarming without affecting flagellation or motility and, because it is of low toxicity to *Proteus* and other bacteria, an evaluation for its use in the clinical laboratory has been advocated (Kopp et al., 1966; Williams, 1973).

Swarms of different strains may fail to penetrate into each other and a sharp line of demarcation is produced between the two swarms (Dienes phenomenon) (Dienes, 1946). In other cases, the swarms may merge into each other without the production of such a line. The occurrence of the line was interpreted to reflect differences in the strains and the absence of the line to signify that the strains were the same. These observations have been exploited for differentiating strains, mostly *P. mirabilis*, in epidemiological studies (Story, 1954). However, strains of different biochemical types may swarm together (Kippax, 1957). Thus, a negative Dienes test (absence of the demarcation line) is less reliable for indicating that the strains are the same than is a positive test (production of the demarcation line) for indicating that the strains are different (France and Markham, 1968; De Louvois, 1969). Results obtained with Dienes tests may fail to correlate with results obtained by bacteriophage typing (Hickman and Farmer, 1976). The production of the line of demarcation appears to be unrelated to the flagellar (H) antigens of the strains (Sourek, 1968; Skirrow, 1969), but appears to depend both on the bacteriocins produced by the swarming strains and on the bacteriocins to which they are sensitive (Senior, 1977). The Dienes test can be usefully employed in epidemiological studies when used in combination with other typing schemes and when its limitations are recognized. The Dienes test has not been tested on *P. myxofaciens* strains.

Another important distinguishing feature of *Proteus* and the other *Proteeae* (*Providencia* and *Morganella*) is their ability to oxidatively deaminate a variety of amino acids, producing keto acids and ammonia (Bernheim et al., 1935; Stumpf and Green, 1944; Singer and Volcani, 1955). Addition of ferric chloride solution to keto acids in aqueous solution produces different colors dependent upon the amino acid from which the keto acid was produced (Singer and Volcani, 1955), and the same colors are produced when ferric chloride solution is added to bacteria grown on nutrient media supplemented with the amino acids. Tests for the differentiation of *Proteus*, *Providencia* and *Morganella* from other *Enterobacteriaceae* that do not produce the deaminases have been developed. Tests for phenylalanine deaminase and for tryptophan deaminase are widely used (Henriksen, 1950; Thibault and Le Minor, 1957).

Bacteriophages lytic for *P. mirabilis* and *P. vulgaris* may be obtained from lysogenic strains or from sewage (Vieu, 1963; Coetzee, 1972). Strains of both *Proteus* species may be differentiated by bacteriophage and, although several schemes, mostly for *P. mirabilis*, have been described, no one scheme has been widely adopted (France and Markham, 1968; Pavlatou et al., 1965; Hickman and Farmer, 1976; Izdebska-Szymona et al., 1971; Schmidt and Jeffries, 1974; Vieu and Capponi, 1965).

Bacteriocins (proticins) may be produced spontaneously or sometimes only after induction with mitomycin C, and bacteriocin typing of *Proteus* strains has been advocated (Cradock-Watson, 1965; Al-Jumaili, 1975; Senior, 1977; Kusek and Herman, 1980). Agreement has not been reached on whether differentiation of the strains should be accomplished on the basis of the inhibitory activity of the bacteriocins produced by the strains under examination, on the basis of sensitivity to a selected set of bacteriocins, or by the use of both methods in combination.

Serotyping of *P. vulgaris* and *P. mirabilis* may be accomplished on the basis of 49 somatic (O) antigens using the simplified scheme of Kauffmann and Perch (Kauffmann, 1966). This scheme includes strains of both species for preparing O antisera. Seventeen of the O antigens are present on *P. vulgaris* strains, 27 on *P. mirabilis* strains, and 5 occur on strains of both species. Three O antigens designated A, B and C, and 11 others designated 100–104 and 200–205, have been defined in other studies but have not been systematically included in an expanded Kauffmann-Perch scheme (Larsson and Olling, 1977; Penner and Hennessy, 1980). Isolates generally agglutinate in antisera against strains of the same species and, therefore, separation of the serovars to provide individual schemes for each species facilitates serotyping (Penner and Hennessy, 1980). The most frequently isolated strains are *P. mirabilis* with O antigens 3, 6 or 10 (Lanyi, 1956; de Louvois, 1969; Larsson and Olling, 1977; Kauffmann, 1966; Penner and Hennessy, 1980).

The number of flagellar (H) antigens in the Kauffmann-Perch scheme is 19. The most common are H antigens 1, 2 and 3. Crossreactions among the H antigens are numerous and complex, and the use of the H antigens in differentiating Proteus strains has been limited essentially to the initial studies of Kauffmann and Perch (Perch, 1948). Capsular (K) antigens (designated C antigens) have been demonstrated for some strains of *P. vulgaris* and *P. mirabilis* (Namioka and Sakazaki, 1959). The antigenic structure of *P. myxofaciens* has not been examined.

Antibodies formed in humans during the course of certain rickettsial infections may react with O antigens of three *Proteus* strains designated X19, X2 and XK. These three strains are used for preparing antisera against *P. vulgaris* 01 and 02 and *P. mirabilis* 03 antigens, respectively. The diagnostic test for antibodies in human sera against these specificities is called the Weil-Felix reaction (see the family *Rickettsiaceae*). Results of this test as an indication of rickettsial infection should be interpreted with caution because of the fact that *Proteus* infections may also evoke antibodies against these antigens and that *P. mirabilis* strains with the 03 antigen are the most frequently isolated *Proteus* strains from human infections.

P. vulgaris and *P. mirabilis* have intrinsic resistance to bacitracin, polymyxin and colistin but are generally susceptible to nalidixic acid. Both species have strains resistant and susceptible to nitrofurantoin. Strains of both species may be either susceptible or resistant to tetracyclines but the proportion of resistant strains is on the increase. The majority of *P. mirabilis* and over 50% of *P. vulgaris* strains are susceptible to chloramphenicol. *P. mirabilis* strains are generally susceptible to penicillins and cephalosporins whereas *P. vulgaris* strains are generally resistant. Most strains of both species are susceptible to the aminoglycosides. Strains of both species may acquire plasmids coding for antibiotic resistances giving rise to marked increase in the resistance to aminoglycosides and/or other antibiotics to which the species is generally susceptible.

Antibiotic susceptibility studies on *P. myxofaciens* have not been reported.

P. mirabilis and *P. vulgaris* may cause primary and secondary infections in man. *P. mirabilis* is much more frequently isolated from clinical specimens than is *P. vulgaris* and is one of the leading pathogens of the human urinary tract. *P. mirabilis* urinary tract infections acquired outside the hospital are often associated with an underlying condition such as diabetes or structural abnormalities of the tract (Wallace and Petersdorf, 1971; Grossberg et al., 1962). *Proteus* urinary tract infections occur more commonly in infection-susceptible hospital patients with predisposing conditions such as catheterization, surgery or urological instrumentation of the tract. Approximately one-quarter of the population are intestinal carriers of *Proteus* (Rustigian and Stuart, 1945) and the patient may become infected with his own flora (autoinfection). Infections may also be contracted through transmission of the bacteria from other patients or from a common reservoir (Dutton and Ralston, 1957; Kippax, 1957). An often-mentioned factor contributing to the pathogenicity of *Proteus* in the urinary tract is the activity of the urease enzyme in producing ammonia and raising the pH (Braude and Siemienski, 1960; MacLaren, 1968; Musher et al., 1975; Griffith et al., 1973; Phillips, 1955). *Proteus* urinary tract infections may give rise to bacteremias that are difficult to treat and often fatal.

Under suitable conditions *Proteus* bacteria may be opportunistic invaders and cause septic lesions at other sites of the body. They have been isolated from infections of wounds, burns, respiratory tract, eyes, ears and throat.

Circumstantial evidence has been cited to implicate *P. mirabilis* as the etiological agents of outbreaks of gastroenteritis resulting from the consumption of contaminated food (Cooper et al., 1941; Cherry et al., 1946) and as the agents causing infantile enteritis (Lanyi, 1956), but their roles as the principal pathogens have been difficult to assess in light of the high carriage rate of *Proteus* in healthy individuals (Carpenter, 1964).

Neonatal umbilical stumps contaminated with *Proteus* bacteria may lead to highly fatal bacteremias and meningitis (Becker, 1962; Burke et al., 1971; Levy and Ingall, 1967; Librach, 1968; Shortland-Webb, 1968).

P. myxofaciens has been isolated only from living and dead gypsy moth larvae (*Porthetria dispar*) but its role as a pathogen of the larvae has not been critically examined.

Proteus strains are widely distributed in nature. *P. mirabilis* is the more common of the two species (Levine and Hoyt, 1945). Both species occur in the intestines of mice, rats, monkeys, raccoons, dogs, cats, cattle, pigs, birds, reptiles and in a large proportion of the human population (Cantu, 1911; Phillips, 1955; Muller, 1972; Wilson and Miles, 1975; Rustigian and Stuart, 1945). The role of *Proteus* in the intestine is not well understood. The bacteria may assist in the hydrolysis of urea although their contribution must be minor in comparison to the large populations of urease-producing anaerobes (Brown et al., 1971; Sabbaj et al., 1970). More important may be their role in the oxidative deamination of amino acids producing keto acids and ammonia (Drasar et al., 1974).

Bacteria of the two species are found in manure, soil and polluted waters where they are thought to have an important function in the decomposition of organic materials (Wilson and Miles, 1975).

Enrichment and Isolation Procedures

Growth of *Proteus* from stool samples is regarded as a nuisance because these bacteria are not generally considered to be infectious agents of the intestine and because their tendency to swarm interferes with the isolation of other bacterial species. Isolation media in the enteric laboratory are therefore designed to inhibit swarming and to preferentially select known pathogens such as *Salmonella* and *Shigella*. Most such media are also suitable for the direct isolation of *Proteus* and are routinely used to isolate *Proteus* from urines and other clinical specimens. However, primary isolation media specific for *Proteus*, *Providencia* and *Morganella* have been designed (Malinowski, 1966; Xilinas et al., 1975; Zarett and Doetsch, 1949).

Tetrathionate or selenite broth are suitable liquid enrichment media when feces are to be examined for *Proteus*. The rate of isolation is increased from 8.2 to 23.6% for *P. mirabilis* and from 0 to 2.7% for *P. vulgaris* when primary plating is preceded by enrichment with tetrathionate (Hynes, 1942; Rustigian and Stuart, 1945).

Maintenance Procedures

Proteus may be maintained on Trypticase soy agar at 4°C with monthly transfers or may be preserved indefinitely by lyophilization. The Enteric Section, Centers for Disease Control, stores cultures at room temperature in tubes of blood agar base or Trypticase soy agar. These tubes are sealed with a cork or rubber stopper and the cultures have remained viable for many years without transfer (J. Farmer and F. Hickman, personal communication).

Differentiation of Proteus *from* Providencia *and* Morganella

Key characteristics for differentiating these three closely related genera are shown in Table 5.41.

Taxonomic Comments

A number of changes have been made since the last edition of the manual in which it was indicated that the genus *Proteus* was composed of five species, namely *P. vulgaris*, *P. mirabilis*, *P. rettgeri*, *P. morganii* and *P. inconstans*. *P. myxofaciens*, on the other hand, was excluded from the genus *Proteus* because it was said to be *Erwinia herbicola* (*Enterobacter agglomerans*). Convincing evidence for major changes in the classification was derived from deoxyribonucleic acid (DNA) relatedness studies (Brenner et al., 1978). Two species were recognized in *Providencia*: cultures previously in biochemical subgroup A were placed in *Providencia alcalifaciens* and those in subgroup B in *Providencia stuartii*. *Proteus rettgeri* was found to be more closely related to the latter two species than to *Proteus vulgaris* or *P. mirabilis* and was, therefore, assigned to the genus *Providencia*. *Proteus morganii* was found to be related to *Proteus* and *Providencia* at levels no greater than to other *Enterobacteriaceae* and was placed in *Morganella*, the genus proposed earlier by Fulton (Fulton, 1943). *Proteus myxofaciens* was included in the genus *Proteus* because of its phenotypic similarity and because of its relatedness by DNA/DNA hybridization to *P. vulgaris* and *P. mirabilis*. Its DNA was only 10% related to DNA from a strain of *Erwinia herbicola*

Table 5.41.
Characteristics differentiating **Proteus, Providencia** *and* **Morganella**[a,b]

Characteristics	*Proteus*	*Providencia*	*Morganella*
Swarming	+	−	−
H_2S production	+	−	−
Gelatin hydrolysis	+	−	−
Lipase (corn oil)	+	−	−
Utilization of citrate (Simmons')	D	+	−
Ornithine decarboxylase	D	−	+
Acid production from:			
Mannose	−	+	+
Maltose	D	−	−
Acid from one or more of the following polyhydric alcohols:			
Inositol, D-mannitol, adonitol, D-arabitol, erythritol	−	+	−

[a] Symbols: see standard definitions.
[b] Temperature of reactions, 36 ± 1°C. All reactions are for 48 h.

Further Reading

Brenner, D. J., J. J. Farmer III, G. R. Fanning, A. G. Steigerwalt, P. Klykken, H. G. Wathen, F. W. Hickman and W. H. Ewing. 1978. Deoxyribonucleic acid relatedness of *Proteus* and *Providencia* species. Int. J. Syst. Bacteriol. *28:* 269–282.

Kauffmann, F. 1966. The Bacteriology of *Enterobacteriaceae*, Williams & Wilkins, Baltimore, pp 333–360.

Rustigian, R. and C. A. Stuart. 1943. Taxonomic relationships in the genus *Proteus*. Proc. Soc. Exp. Biol. Med. *53:* 241–243.

Rustigian, R. and C. A. Stuart. 1945. The biochemical and serological relationships of the organisms of the genus *Proteus*. J. Bacteriol. *49:* 419–436.

Williams, F. D. 1978. Nature of the swarming phenomenon in *Proteus*. Annu. Rev. Microbiol. *32:* 101–122.

List of the species of the genus Proteus

1. **Proteus vulgaris** Hauser 1885, 12.[AL]
vul.ga'ris. L. adj. *vulgaris* common.
Morphological characteristics are as described for the genus. Other characteristics are listed in Tables 5.41 to 5.43.
Some strains are hemolytic on blood agar.
Less frequently found in clinical specimens than *P. mirabilis*.
Generally resistant to penicillins and cephalosporins.
The mol% of the DNA is 39.3 ± 1.2% (T_m) (Falkow et al., 1962).
Type strain: ATCC 13315.

2. **Proteus mirabilis** Hauser 1885, 34.[AL]
mi.ra'bi.lis. L. adj. *mirabilis* wonderful.
Morphological characteristics are as described for the genus. Other characteristics are listed in Tables 5.41 to 5.43.
Some strains are hemolytic on blood agar.
More frequently found in clinical specimens than *P. vulgaris*.
Most common site of infection is the urinary tract. Generally susceptible to ampicillin and cephalosporins.
The mol% of the DNA is 39.3 ± 1.4% (T_m) (Falkow et al., 1962).
Type strain: ATCC 29906.

3. **Proteus myxofaciens** Cosenza and Podgwaite 1966, 188.[AL]
myx.o.fac'i.ens. Gr. fem. n. *myxa*, slime; M.L. masc. n. *faciens* producing; *myxofaciens* slime-producing (bacteria).
Morphological characteristics are as described for the genus. Other characteristics are listed in Tables 5.41 to 5.43. Only one strain studied in detail. Thin film of growth on solid media. Produces highly viscous slime. Hemolytic on blood agar.
Isolated from living and dead gypsy moth larvae (*Porthetria dispar* L).
Type strain: ATCC 19692.

Table 5.42.
Differential characteristics of the species of the genus **Proteus**[a]

Characteristics	1. *P. vulgaris*	2. *P. mirabilis*	3. *P. myxofaciens*[b]
Indole production	+	−	−
Ornithine decarboxylase	−	+	−
Acid from:			
Maltose	+	−	+
α-Methylglucoside	d	−	+
D-Xylose	d	+	−
Tyrosine clearing	+	+	−
Slime production, 25°C in TSB[c]	−	−	+

[a] Temperature of reactions, 36 ± 1°C. All reactions are for 48 h. For symbols see standard definitions
[b] Reactions based on study of only one strain (ATCC 19692).
[c] TSB, Trypticase soy broth.

Table 5.43.
Other characteristics of the species of the genus **Proteus**[a]

Characteristics	1. *P. vulgaris*	2. *P. mirabilis*	3. *P. myxofaciens*[b]
Phenylalanine deaminase	+	+	+
Urease	+	+	+
NO_3^- reduced to NO_2^-	+	+	+
Motility	+	+	+
Swarming	+	+	+
Gelatin liquefaction (22°C)	+	+	+
H_2S production (triple-sugar iron agar)	+	+	+ (3–4 days)
Growth in KCN	+	+	+
Acid from glucose	+	+	+
Gas from glucose	+	+	+
Methyl red test	+	+	+
Voges-Proskauer test	−	d	+
Citrate utilization (Simmons')	d	d	+
Tartrate utilization (Jordan)	+	d	+
Acetate utilization	d	d	−
Lipase activity (corn oil)	d	+	+
Deoxyribonuclease (25°C)	d	d	−
Oxidase test	−	−	−
ONPG hydrolysis[c]	−	−	−
Pectate liquefaction	−	−	−
Malonate utilization	−	−	−
Amino acid decarboxylases (Møller):			
Lysine decarboxylase	−	−	−
Arginine dihydrolase	−	−	−
Acid production from:			
Sucrose	+	d	+
Trehalose, glycerol	d	+	+
Salicin, esculin	d	−	−
Lactose, L-arabinose, raffinose, L-rhamnose, cellobiose, mannose, melibiose, mucate, inositol, D-mannitol, adonitol, D-arabitol, S-sorbitol, dulcitol, erythritol	−	−	−

[a] Temperature of reactions, 36 ± 1°C unless otherwise noted. All reactions are for 48 h except where otherwise noted. For symbols see standard definitions.
[b] Based on study of only one strain (ATCC 19692).
[c] ONPG, *o*-nitrophenyl-β-D-galactopyranoside.

Genus XII. **Providencia** *Ewing 1962, 96*[AL]

JOHN L. PENNER

Pro.vi.den'ci.a. M.L. fem. n. *Providencia* named after the city of Providence, Rhode Island, U.S.A.

Straight rods, 0.6–0.8 × 1.5–2.5 μm, conforming to the general definition of the family *Enterobacteriaceae*. Gram-negative. **Motile** by peritrichous flagella. **Swarming does not occur.** Facultatively anaerobic. **Oxidatively deaminate phenylalanine and tryptophan. Produce acid from one or more of the following polyhydric alcohols:** inositol, D-mannitol, adonitol, D-arabitol, erythritol. **Acid is produced from mannose.** Indole-positive. **Citrate** (Simmons') and tartrate (Jordan) are utilized. Isolated from diarrhetic stools, urinary tract infections, wounds, burns and bacteremias. The mol% G + C of the DNA is 39–42% (Falkow et al., 1962).

Type species: *Providencia alcalifaciens* Ewing 1962, 96.

Further Descriptive Information

Providencia strains, like those of *Proteus* and *Morganella*, deaminate phenylalanine, and at least some strains of the genus deaminate other amino acids (Singer and Volcani, 1955). Like other *Proteeae*, *Providencia* strains decompose tyrosine to produce a clearing on the agar media in which the insoluble amino acid is incorporated (Sheth and Kurup, 1975), and produce a reddish-brown pigment when cultured on nutrient agar containing 5% tryptophan (Polster and Svobodova, 1964). *Providencia* differs from other *Proteeae* by being able to produce acid from inositol and straight-chain tetra-, penta- or hexahydroxy alcohols, and the species of *Providencia* are differentiated on the basis of their reactions on these substrates. Yellow-orange-centered colonies are produced by *Providencia* on deoxycholate citrate agar (Cook, 1948; Buttiaux et al., 1954). The color is apparently caused by the precipitation of ferric hydroxide as a result of the alkalinity produced by the growth of the bacteria on the medium (Catsaras et al., 1965).

Urease is produced characteristically by strains of only one species, *P. rettgeri*. The proportion of urease-positive strains of *P. stuartii* has been estimated to be 15% (Brenner et al., 1978), although subsequent calculations based on a larger number of strains indicate 6–10% (Penner et al., 1979). The urease enzyme of at least some *P. stuartii* strains is encoded on a transferable plasmid (Grant et al., 1981). The presence of the plasmid in endemic strains of some hospitals could be expected to cause variations among the hospitals in the frequency of isolation of urease-positive *P. stuartii*.

Providencia bacteriophages have lytic activity on *Providencia* and *Proteus* strains but not on *Morganella* strains (Coetzee, 1963). The phages may be isolated from sewage and from lysogenic strains. A scheme consisting of 12 selected bacteriocins may be used to differentiate strains of *P. alcalifaciens* and *P. stuartii* (Al-Jumaili and Fenwick, 1978).

Thermostable somatic (O) antigens, thermolabile flagellar (H) antigens and capsular (K) antigens occur in *Providencia*. The original antigenic scheme was for two species (*P. alcalifaciens* and *P. stuartii*) and consisted of 56 O antigens, 28 H antigens and 2 K antigens (Ewing et al., 1954). For differentiation of strains on the basis of the O antigens the schemes have been extended and separated according to species, so that, currently, 46 O antigens for *P. alcalifaciens* and 17 O antigens for *P. stuartii* may be identified (Penner et al., 1979a, b). The original schemes for *P. rettgeri* listed 34 O antigens and 26 H antigens (Namioka

and Sakazaki, 1958). New serovars have been isolated and the number of O antigens now recognized is 93 (Penner and Hennessey, 1979).

The practice of listing *P. rettgeri* with *Proteus vulgaris* and *Morganella morganii* in the "indole-positive *Proteus* group," and *P. alcalifaciens* and *P. stuartii* in the "Providence" group, in antimicrobial agent-susceptibility studies has tended to obscure significant species differences in susceptibility. Generally, strains of *P. alcalifaciens* are more susceptible than are *P. stuartii* and *P. rettgeri* to penicillins, cephalosporins and aminoglycosides (Overturf et al., 1974; Penner and Preston, 1980). The most resistant *Providencia* strains are found in the species *P. stuartii*. Amikacin is often effective against *P. stuartii* strains that are resistant to other antibiotics.

The urinary tract of the catheterized or compromised patient is the most common site of *P. stuartii* and *P. rettgeri* infections. Strains of the two species may also produce wound and burn infections and bacteremias. The rise in medical importance of these organisms is associated with their tendency to cause nosocomial infections and with their marked resistance to numerous antibiotics.

P. alcalifaciens strains are generally isolated from stool specimens taken from patients with diarrhea. The most common serotype isolated is 0:3. Whether these bacteria, particularly of this serotype, are indeed the causative agents of the diarrheas as claimed (Carpenter, 1964) or whether they are commensals that flourish during infections caused by viral or other bacterial agents remains to be determined.

Providencia isolates recovered in studies on *Proteus* indicate that there is some overlapping of habitats between the two genera. *Providencia* strains are rarely isolated from intestines of healthy individuals by methods routinely employed in examining fecal specimens (Singer and Bar-Chay, 1954). Rigorous examinations have not been conducted to determine if this reflects a genuinely low incidence or if it reflects small bacterial populations that are detectable only with special media.

Enrichment and Isolation Procedures

Media used in the clinical laboratory for isolation of *Enterobacteriaceae* may be used to isolate *Providencia*. Tetrathionate or selenite broths may be used for enrichment. Media for the specific isolation of *Providencia* have not been reported, but the medium of Malinowski (1966) should be considered because differentiation from other *Enterobacteriaceae* does not depend upon hydrolysis of urea.

Maintenance Procedures

Providencia strains may be maintained on trypticase soy agar at 4°C with monthly transfers or may be preserved indefinitely by lyophilization. The Enteric Section, Centers for Disease Control, stores cultures at room temperature in tubes of blood agar base or trypticase soy agar. These tubes are sealed with a cork or rubber stopper and the cultures have remained viable for many years without transfer (J. Farmer and F. Hickman, personal communication).

Taxonomic Comments

Major changes in the classification of members of the tribe *Proteeae* since the last edition of the *Manual* have led to the emergence of the genus *Providencia*. Two species of the genus, *P. alcalifaciens* and *P. stuartii*, were previously included in one species of the genus *Proteus* (*Proteus inconstans*) or were often grouped together and called the "Providence" strains. Bacteria that are now known to be urease-positive strains of *P. stuartii* were included along with typical *P. rettgeri* in *Proteus rettgeri*. The new classification in the present *Manual* was introduced because it was confirmed through DNA/DNA hybridization studies that these bacteria were a group distinct from other *Proteeae* (Brenner et al., 1978). In *Proteus inconstans* two distinct groups that corresponded to the biochemical types (subgroups A and B) were recognized. Since there is doubt about the validity of the epithet *inconstans* introduced by Ornstein (1921) to indicate variability in the fermentation of glucose by a bacterium for which no subculture of the original strain exists, the validity published epithets *alcalifaciens* and *stuartii* were selected, the former for subgroup A strains and the latter for subgroup B strains. This was in accordance with proposals previously published (Ewing, 1962).

Proteus rettgeri was also found to consist of two groups on the basis of DNA/DNA hybridization studies. One group consisted of typical *P. rettgeri*. The other group consisted of urease-positive strains of *P. stuartii* and were reassigned to that species.

Interpreting data from earlier studies may cause problems because sometimes it is not clear, in light of the new classification, to which species the bacteria under study actually belonged. A case in point concerns the data on the mol% G + C content of DNA. It is not certain whether the value reported for *Proteus rettgeri* (39 ± 1.5) is for a typical strain or for a urease-positive *P. stuartii* strain, or if the value reported for *Proteus inconstans* (41.5 ± 0.6) is for *P. alcalifaciens* or *P. stuartii* (Lautrop, 1974; Falkow et al., 1962). Similar problems may arise in other studies in which the earlier classifications were used.

Further Reading

Brenner, D. J., J. J. Farmer III, G. R. Fanning, A. G. Steigerwalt, P. Klykken, H. G. Wathen, F. W. Hickman and W. H. Ewing. 1978. Deoxyribonucleic acid relatedness of *Proteus* and *Providencia* species. Int. J. Syst. Bacteriol. *28:* 269–282.

Ewing, W. H. 1962. The Tribe *Proteeae:* its nomenclature and taxonomy. Int. Bull. Bacteriol. Nomencl. Taxon. *12:* 93–102.

Differentiation of the species of the genus Providencia

Table 5.44 presents characteristics for differentiation of the three species of *Providencia*.

Table 5.44.
Differential characteristics of the species of the genus **Providencia**[a]

Characteristics	1. *P. alcalifaciens*	2. *P. stuartii*	3. *P. rettgeri*
Urease production	−	d	+
Acid production from:			
Inositol	−	+	+
D-Mannitol	−	d	+
Adonitol	+	−	+
D-Arabitol	−	−	+
Erythritol	−	−	d
Trehalose	−	+	−

[a] Temperature of reactions, 36 ± 1°C. All reactions are for 48 h. For symbols see standard definitions.

List of the species of the genus **Providencia**

1. **Providencia alcalifaciens** (De Dalles Gomes 1944) Ewing 1962, 96.[AL] (*Eberthella alcalifaciens* de Salles Gomes 1944, 183; *Proteus inconstans* (Ornstein 1921) Shaw and Clarke 1955, 155.)

al.cal.i.fac'i.ens. Fr. n. *alcali* alkali; L. v. *facere* to do, make; L. part. adj. *faciens* making; M.L. part. adj. *alcalifaciens* alkali-producing.

The characteristics are as described for the genus and as listed in Tables 5.44 and 5.45.

Most strains are susceptible to penicillins and cephalosporins.

Generally isolated from diarrhetic stools, particularly from children, but the role in disease production is not known. The most frequently isolated strains are serovar 0:3.

Type strain: ATCC 9886.

2. **Providencia stuartii** (Buttiaux et al., 1954) Ewing 1962, 96.[AL] (*Proteus stuartii* Buttiaux, Osteux, Fresnoy and Moriamez 1954, 385; *Proteus inconstans* (Ornstein 1921) Shaw and Clarke 1955, 155.)

stu.ar'ti.i. M.L. gen. n. *stuartii* of Stuart; named after C. A. Stuart, bacteriologist at Providence, Rhode Island, U.S.A.

The characteristics are as described for the genus and as listed in Tables 5.44 and 5.45.

Many strains are resistant to penicillins and cephalosporins. Some strains are resistant to gentamicin and kanamycin. Some exceptional strains are resistant to most antibiotics in current use.

Isolated most often from urine specimens of hospitalized and catheterized patients. Less frequently isolated from wounds, burns and bacteremias. May cause nosocomial infections. Rarely isolated from stool specimens.

Type strain: ATCC 29914.

3. **Providencia rettgeri** (Hadley, Elkins and Caldwell 1918) Brenner, Farmer, Fanning, Steigerwalt, Klykken, Wathen, Hickman and Ewing 1978, 269.[AL] (*Bacterium rettgeri* Hadley, Elkins and Caldwell 1918, 180; *Proteus rettgeri* (Hadley et al. 1918) Rustigian and Stuart 1943, 242.)

rett'ge.ri. M.L. gen. n. *rettgeri* of Rettger; named after L. F. Rettger, the American bacteriologist who first isolated the organism in 1904.

The characteristics are as described for the genus and as listed in Tables 5.44 and 5.45.

Many strains are resistant to penicillins and cephalosporins, but the strains are generally not as resistant as *P. stuartii* strains.

Generally isolated from urine specimens of hospitalized and catheterized patients. Less frequently isolated from other sites. May cause nosocomial infections. Rarely isolated from stool specimens.

Type strain: ATCC 29944.

Table 5.45.
Other characteristics of the species of the genus **Providencia**[a]

Characteristics	1. *P. alcalifaciens*	2. *P. stuartii*	3. *P. rettgeri*
Phenylalanine deaminase	+	+	+
Indole production	+	+	+
Nitrates reduced to nitrites	+	+	+
Motility, 36°C	+	d	+
Growth in KCN	+	+	+
Methyl red test	+	+	+
Voges-Proskauer test	−	−	−
Citrate utilization (Simmons')	+	+	+
Tartrate utilization (Jordan)	+	+	+
Acetate utilization	d	d	d
Lipase activity (corn oil)	−	−	−
Oxidase test	−	−	−
β-Galactosidase (ONPG test)	−	−	−
Pectate liquefaction	−	−	−
Tyrosine clearing	+	+	+
Malonate utilization	−	−	−
Amino acid decarboxylases (Møller)			
Lysine decarboxylase	−	−	−
Arginine dihydrolase	−	−	−
Ornithine decarboxylase	−	−	−
Gelatin liquefaction (22°C)	−	−	−
H_2S production (triple-sugar iron agar	−	−	−
Acid production from:			
Glucose, mannose	+	+	+
Sucrose, glycerol	d	d	d
Esculin	−	d	−
D-Xylose, salicin, L-rhamnose	−	−	d
Lactose, L-arabinose, raffinose, maltose, cellobiose, α-methylglucoside, melibiose, mucate, dulcitol, D-sorbitol	−	−	−
Gas from glucose	d	−	d

[a] Temperature of reactions, 36 ± 1°C unless otherwise noted. All reactions are for 48 h. For symbols see standard definitions.

Genus XIII. **Morganella** *Fulton 1943, 81*[AL]

JOHN L. PENNER

Mor.ga.nel'la. M.L. dim. ending *-ella*; M.L. fem. n. *Morganella* named after H. de R. Morgan, who first studied the organism.

Straight rods, 0.6–0.7 μm in diameter and 1.0–1.7 μm in length, conforming to the general definition of the family *Enterobacteriaceae.* Gram-negative. **Motile** by means of peritrichous flagella, but some strains do not form flagella above 30°C. After 48 h on 1% agar media at 22°C growth may spread to form a surface film. **Swarming does not occur.** Facultatively anaerobic. **Deaminate phenylalanine and tryptophan oxidatively. Urease-positive. Indole-positive. Ornithine is decarboxylated.** A few carbohydrates can be fermented. **Produce acid from mannose.** Utilize Jordan tartrate but not Simmons' citrate. Occur in the feces of humans, dogs, other mammals and reptiles. Opportunistic secondary invaders, isolated from bacteremias, respiratory tract, wound and urinary tract infections. The mol% G + C of the DNA is 50 (T_m).

Type species: Morganella morganii (Winslow et al. 1919) Brenner et al. 1978, 269.

Further Descriptive Information

Until recently, the members of *Morganella* were classified as *Proteus* and were thus considered in the light of their membership in that genus rather than as a separate group. Like *Proteus*, *Morganella* strains can be cultured on laboratory media used for enteric bacteria but some strains may not form flagella above 30°C (Coetzee and De Klerk, 1964). After 48 h on 1% agar media at 22°C, growth may spread to form a film (Rauss, 1936; Coetzee and De Klerk, 1964) and the culture may consist of semifilamentous forms resembling those of *Proteus* (Rauss, 1936); however, swarming on 1.5% agar (with cycles of migration and consolidation typical of *Proteus*) has not been demonstrated (Sevin and Buttiaux, 1939). Some *Morganella* strains are hemolytic on blood agar.

Like *Proteus* and *Providencia*, *Morganella* strains produce urease and phenylalanine deaminase; however, the *Morganella* enzymes are serologically unrelated to those of the other two genera (Guo and Liu, 1965; Smit and Coetzee, 1967), and the urease has other properties which differ markedly from those of the ureases of *Proteus* and *Providencia* (Richard, 1965; Rosenstein et al., 1981). *Morganella* strains also decompose tyrosine to produce a clearing on media containing the insoluble amino acid (Sheth and Kurup, 1975). *Morganella* strains also produce a reddish-brown pigment when cultured on nutrient media supplemented with 5% tryptophan (Polster and Svobodova, 1964). Unlike *Proteus* and *Providencia*, *Morganella* strains are noted for their inability to ferment carbohydrates. Glucose and mannose are the only sugars from which *Morganella* strains typically produce acid. Trehalose and glycerol are fermented by some strains (Hickman et al., 1980) and lactose-positive strains have been isolated occasionally (Sutter and Foecking, 1962; Tierno and Steinberg, 1975); the ability to ferment lactose is plasmid-encoded (Le Minor and Coynault, 1976). Unlike *Proteus* and *Providencia*, *Morganella* strains do not produce a red color on lysine iron agar (Edwards and Ewing, 1972). Typically, *Morganella* strains decarboxylate only ornithine, but a few strains decarboxylate both ornithine and lysine, and a few decarboxylate neither (Hickman et al., 1980); lysine decarboxylase in *Morganella* is plasmid-encoded (Cornelis et al., 1981). *Morganella strains* require niacin and pantothenate for growth (Pelczar and Porter, 1940).

Morganella bacteriophages do not generally attack *Proteus* and *Providencia* strains (Coetzee, 1963). Twelve lytic patterns have been found among 26 *Morganella* strains using seven bacteriophages (Schmidt and Jeffries, 1974). The activities of 12 *Morganella* bacteriocins (morganocins) are detectable on MacConkey agar but not on nutrient agar (Coetzee, 1967).

The original antigenic scheme based on somatic (O) and flagellar (H) antigens (Rauss and Vörös, 1959) has been extended to 42 serogroups and 75 serovars (Rauss et al., 1975). The O antigens can be determined by passive hemagglutination (Penner and Hennessy, 1979).

Morganella strains are generally resistant to colistin, erythromycin, penicillin, ampicillin and cephalothin, and are generally susceptible to nalidixic acid, carbenicillin, the aminoglycosides and chloramphenicol. There is much variation among the strains in susceptibility to tetracyclines and sulfonamides.

Morganella was once considered to be a cause of diarrhea (Morgan, 1906; Tribondeau and Fichet, 1916; Magheru, 1923; Thjøtta, 1920; Rauss, 1936) because it was found as the predominant species in diarrhetic stools and because other known pathogens (*Salmonella*, *Shigella*) were not present. Reports of this type have been lacking in recent years, however, and firm evidence for an etiological role in enteritis has not been forthcoming. There is considerably more evidence for a pathogenic role in urinary tract infections, particularly for those of nosocomial origin (Sevin and Buttiaux, 1939; Lanyi, 1957; Von Graevenitz and Spector, 1969; McMillan, 1972). It is an opportunistic, secondary invader rather than a primary pathogen at other sites and has been isolated from blood, sputa and pus from patients with bacteremias, respiratory tract and wound infections.

The habitat of *Morganella* has not been examined systematically, but it has been isolated from the intestines of humans, dogs, other mammals and reptiles (Phillips, 1955; Müller, 1972).

Enrichment and Isolation Procedures

Media for primary isolation of *Enterobacteriaceae* are usually used for isolating *Morganella.* The culturing of feces in tetrathionate or selenite broth prior to plating on enteric media increases the rate of *Morganella* isolations from 1.8 to 10% in studies on human intestinal carriage (Rustigian and Stuart, 1945).

Maintenance Procedures

Morganella strains may be maintained on trypticase soy agar with monthly transfers or may be preserved indefinitely by lyophilization. The Enteric Section, Centers for Disease Control, stores cultures at room temperature in tubes of blood agar base or trypticase soy agar. These tubes are sealed with a cork or rubber stopper and the cultures have remained viable for many years without transfer (J. Farmer and F. Hickman, personal communication).

Differentiation of the genus **Morganella** *from other genera*

See the genus *Proteus*, Table 5.41, for characteristics that can be used to differentiate *Morganella* from other related genera of *Enterobacteriaceae.*

Taxonomic Comments

A major change since the last edition of the *Manual* has been the transfer of this group from the genus *Proteus* to the genus *Morganella.* Rauss (1936) concluded that the organism "belongs taxonomically to the genus *Proteus*," and Yale (1939) attributed the name *Proteus morganii* to him. Yale, however, not Rauss, was cited as the author in the eighth edition of the *Manual* because it was pointed out that Rauss had not actually published the name (Lessel, 1971). The inclusion in the genus *Proteus* received further support because the organisms, like *P. vulgaris* and *P. mirabilis*, hydrolyzed urea (Rustigian and Stuart, 1943). The major criteria supporting the elevation of *P. morganii* to generic rank are that the DNA contains 50 mol% G + C, which is

similar to the DNA base composition of *Escherichia coli* and *Salmonella* rather than *Proteus*, and that DNA/DNA hybridization studies show that the organisms are related at only a 20% level to most enteric bacteria and at not more than 20% to *Proteus* (Brenner et al., 1978).

Further Reading

Brenner, D. J., J. J. Farmer III, G. R. Fanning, A. F. Steigerwalt, P. Klykken, H. G. Wathen, F. W. Hickman and W. H. Ewing. 1978. Deoxyribonucleic acid relatedness of *Proteus* and *Providencia* species. Int. J. Syst. *Bacteriol 28:* 269–282.

Hickman, F. W., J. J. Farmer III, A. E. Steigerwalt and D. J. Brenner. 1980. Unusual groups of *Morganella* ("Proteus") *morganii* isolated from clinical specimens: lysine-positive and ornithine-negative biogroups. J. Clin. Microbiol. *12:* 88–94.

Rauss, K. and S. Vörös. 1959. The biochemical and serological properties of *Proteus morganii*. Acta Microbiol. Acad. Sci. Hung. *6:* 233–246.

Rustigian, R. and C. A. Stuart. 1943. Taxonomic relationships in the genus *Proteus*. Proc. Soc. Exper. Biol. Med. *53:* 241–243.

List of the species of the genus **Morganella**

1. **Morganella morganii** (Winslow, Kligler and Rothberg 1919) Brenner, Farmer, Fanning, Steigerwalt, Klykken, Wathen, Hickman and Ewing 1978, 269.[AL] (*Bacillus morgani* (sic) Winslow, Kligler and Rothberg 1919, 481; *Proteus morganii* (Winslow et al.) Yale 1939, 435.)

mor.ga'ni.i. M.L. gen. n. *morganii* of Morgan; named after H. de R. Morgan, a British bacteriologist who first studied the organism.

The description is the same as that for the genus. See Table 5.46 for other characteristics.

Occur in the feces of humans, dogs, other mammals and reptiles. Opportunistic human pathogens.

The mol% G + C of the DNA is 50 (Falkow et al., 1962).

Type strain: ATCC 25830.

Table 5.46.
Characteristics of **Morganella morganii**[a]

Test	Reaction or Result
Phenylalanine deaminase	+
Urease	+
Indole	+
Growth in KCN	+
Amino acid decarboxylases (Møller):	
Ornithine decarboxylase	+
Lysine decarboxylase	−
Arginine dihydrolase	−
Methyl red test	+
Voges-Proskauer test	−
NO_3^- reduced to NO_2^-	d
Tyrosine clearing	+
Oxidase test	−
ONPG hydrolysis[b]	−
Deoxyribonuclease	−
Lipase	−
Tartrate utilization (Jordan)	+
H_2S production (triple-sugar iron agar)	−
Motility	+
Gelatin liquefaction	+
Utilization of citrate (Simmons')	−
Utilization of acetate or malonate	−
Acid production from:	
Glucose, mannose	+
Trehalose	d
Lactose, sucrose, L-arabinose, raffinose, L-rhamnose, D-xylose, cellobiose, α-methyl-glucoside, melibiose, salicin, esculin, mucate	−
Gas from glucose	d

[a] Temperature of reactions, 36 ± 1°C. All reactions are for 48 h. Symbols: see standard definitions.
[b] ONPG, *o*-nitrophenyl-β-galactopyranoside.

Genus XIV. **Yersinia** *Van Loghem 1944, 15.*[AL]

HERVE BERCOVIER AND HENRI H. MOLLARET

Yer.si'ni.a. M.L. fem. n. *Yersinia* named for the French bacteriologist A. J. E Yersin, who first isolated the causal organisms of plague in 1894.

Straight rods to coccobacilli, 0.5–0.8 μm in diameter and 1–3 μm in length. Endospores are not formed. Capsules are not present, but an envelope occurs in *Y. pestis* strains grown at 37°C or in cells from in vivo samples. Gram-negative. **Nonmotile at 37°C, but motile with peritrichous flagella when grown below 30°C, except for *Y. pestis* which is always nonmotile.** Growth occurs on ordinary nutrient media. Colonies on nutrient agar are translucent to opaque, 0.1–1.0 mm in diameter after 24 h. Optimum temperature, 28–29°C.

Facultatively anaerobic, having both a respiratory and a fermentative type of metabolism. Oxidase-negative. Catalase-positive. Nitrate is reduced to nitrite with a few exceptions in specific biovars. Glucose and other carbohydrates are fermented with acid production **but little or no gas. Phenotype characteristics are often temperature-dependent,** and usually more characteristics are expressed by cultures incubated at 25–29°C than at 35–37°C. The enterobacterial common antigen is expressed by all species investigated. Occur in a broad spectrum of habitats (live and inanimate), with some species adapted to specific hosts. The mol% G + C of the DNA is 46–50 (T_m, Bd).

Type species: *Yersinia pestis* (Lehmann and Neumann 1896) Van Loghem 1944, 15.

Further Descriptive Information

Cells of *Yersinia* species are small, coccoid-shaped Gram-negative bacilli that resemble cells of *Pasteurellaceae* rather than of *Enterobacteriaceae*. Pleomorphism occurs depending on the type of medium used and the temperature of incubation. Rods, coccobacilli, and small chains of 4 or 5 elements (especially in liquid media) can be seen in a Gram stain, which reveals a more pronounced tendency to bipolar staining in *Y. pestis* than in the other species. No spores or specific inclusions are formed. No definite capsules occur, but *Y. pestis* displays an envelope that might be taken for a capsule when cultured in proper media (Burrows, 1963) incubated at 37°C, or when stained in samples taken from live hosts (mice, guinea pigs, humans). L forms have been described for *Y. enterocolitica* (Pease, 1979).

All *Yersinia* species are nonmotile when incubated at 37°C but motile at 22–29°C, except *Y. pestis*, which is never motile. Fresh isolates of *Y. enterocolitica* and *Y. pseudotuberculosis* may require a few subcultures to express their motility. Motile cells have 2–15 peritrichous flagella characterized by a long wavelength (Nilehn, 1969).

Yersinias do not differ from other *Enterobacteriaceae* in their fine structure and overall cell wall composition. Lipopolysaccharides (O antigens) have been isolated and characterized (Davies, 1958; Rische et al., 1973). The whole-cell lipid composition of all *Yersinia* species investigated exhibits a pattern shared with other *Enterobacteriaceae* (Tornabene, 1973, Jantzen and Lassen, 1980).

Yersinia species grow on nutrient agar without enrichment. A small colony diameter differentiates yersinias from all other *Enterobacteriaceae*. After incubation for 24–30 h at 30 or 37°C, *Y. pestis* forms minute colonies (0.1 mm) that can be discerned only with difficulty by the naked eye. After 48 h their diameter increases to 1.0–1.5 mm. The colonies are slightly opaque, butyrous, smooth, round, and have somewhat irregular edges. The use of enriched media (serum, blood, yeast extract) does not dramatically improve the growth, and after 48 h the colony sizes are similar to those found on nutrient agar. All other *Yersinia* species grown on nutrient agar at 25–37°C produce visible colonies in 24 h. The colonies reach a diameter of 1.0–1.5 mm after 24–30 h, and 2.0–3.0 mm after 48 h. After 18 h they are translucent, smooth and round with irregular edges, but after 48 h the centers become elevated and the edges become more regular, producing a "chinese hat" shape. When cultured for 48 h, all *Yersinia* species dissociate into small (0.5 mm) and large colonies (2 mm). This phenomenon appears to depend on the medium used (Bercovier et al., 1979).

Growth is moderate in liquid media: incubation of yersinias for 48 h will yield the same turbidity that occurs in 18 h with other *Enterobacteriaceae*. When grown in nutrient broth *Y. pestis* forms a deposit at the bottom of the tube and the supernatant remains relatively clear; this is followed by the appearance of a pellicle, which in turn disintegrates to form flocculent masses and a larger deposit. This phenomenon is attenuated in peptone water. *Y. pseudotuberculosis* occasionally grows in a manner similar to that of *Y. pestis*. All other *Yersinia* species give uniform turbidity in nutrient broth and in peptone water.

Y. pestis and *Y. pseudotuberculosis* give variable growth responses on MacConkey agar. All the other species grow well on this medium, with colonies reaching a size similar to that observed on nutrient agar. On salmonella-shigella agar incubated at 25°C *Y. pestis* hardly grows at all, whereas all the other species produce pin-point colonies in 24–30 h. When incubated on this medium at 37°C, *Y. enterocolitica* is only partially inhibited, whereas all other species are severely inhibited (Bottone, 1977; Nilehn, 1969; Bercovier et al., 1979).

All *Yersinia* species except *Y. pestis* can grow at 25°C on synthetic mineral-salt media with various carbohydrates as the energy source (Burrows and Gillet, 1966; Bercovier et al., 1979). *Y. pestis* requires L-methionine and L-phenylalanine. When incubated at 37°C on synthetic mineral-salt media all *Yersinia* species become auxotrophic, and the addition of at least biotin and thiamine is necessary to promote growth (Burrows and Gillet, 1966). The growth of *Y. pestis* on such media is enhanced by the addition of L-isoleucine, L-valine, glycine, L-threonine, and reducing agent, and by incubation in a CO_2-enriched atmosphere (Brubaker, 1972). Virulent strains of *Y. pestis* require Ca^{2+} or ATP for growth at 37°C but not at 25°C (Zahorchak et al., 1979). This temperature-dependent requirement for Ca^{2+} has also been described for some virulent strains of *Y. pseudotuberculosis* and *Y. enterocolitica*.

All *Yersinia* species grow at temperatures of 4–42°C, with an optimum temperature of 28–29°C. *Y. pestis* and *Y. pseudotuberculosis* tolerate a pH range of 5.0–9.6; other *Yersinia* species can grow in a pH range of 4.0–10.0. The optimum pH for all species is 7.2–7.4.

Yersinia species can grow in peptone water without the addition of NaCl. *Y. pestis* and *Y. pseudotuberculosis* tolerate up to 3.5% NaCl, and the other species can tolerate up to 5% NaCl. *Y. pseudotuberculosis* is the only species which grows well on media containing 0.06% tellurite (Brzin, 1968).

Yersinias do not differ significantly from other *Enterobacteriaceae* in their general metabolism (Brubaker, 1972). They produce acid during fermentation of glucose. *Y. enterocolitica*, *Y. frederiksenii* and *Y. intermedia* produce acetoin when incubated at 28°C, whereas this characteristic is variable for *Y. ruckeri* and is always absent in *Y. pestis* and *Y. pseudotuberculosis*. No species produces acetoin at 37°C.

The main physiological and biochemical characteristics of the various *Yersinia* species are given in Tables 5.48 and 5.49. Yersinias ferment carbohydrates without gas production; this characteristic is constant for *Y. pestis* and *Y. pseudotuberculosis*, but other species may produce a few bubbles after 2 or 3 days at 28°C. Because the optimum growth temperature of yersinias is 28–29°C, some biochemical activities are often temperature-dependent (cellobiose and raffinose fermentation, ornithine decarboxylase, ONPG (*o*-nitrophenyl-β-D-galactopyranoside) hydrolysis, indole production, and the Voges-Proskauer reaction) and are more constantly expressed at 28°C rather than at 37°C. all species except *Y. intermedia* reduce nitrate to nitrite by a type B nitrate reductase; *Y. intermedia* strains have either a type A nitrate reductase, like most *Enterobacteriaceae*, or a type B reductase. The ONPG activity of yersinias does not correspond to a true β-galactosidase, but only to an ONPG-ase (Le Minor et al., 1977). In addition to the characteristics given in Tables 5.48 and 5.49, *Yersinia* species are able to attack polypectate in 5–7 days and starch in 3–7 days. Yersinias are neither hemolytic nor proteolytic, except *Y. ruckeri*, which liquefies gelatin, and some strains of *Y. pestis* which have fibrinolytic and coagulase activity linked to the production of Pesticin I. Lecithinase activity in *Y. enterocolitica* is strain-dependent. *Y. pseudotuberculosis*, *Y. enterocolitica* and *Y. ruckeri* strains have a lipase that is active on corn oil, but only *Y. intermedia*, *Y. frederiksenii* and *Y. enterocolitica* biovar 1 express a lipase-esterase that is active on Tween 80.

Transformation of auxotrophic strains of *Y. enterocolitica* by prototrophic strains using the Juni-Janik technique has been reported (Callahan and Koroma, 1979). F lac$^+$ episomes from *E. coli* have been transferred to *Y. pestis* (Martin and Jacob, 1962), to *Y. pseudotuberculosis* (Lawton et al., 1968b) and to *Y. enterocolitica* (Cornelis and Colson, 1975), but usually with a low frequency (10^{-4}–10^{-6}). This has allowed chromosomal mapping of *Y. pseudotuberculosis* (Lawton and Stull, 1971; McMahon, 1973). Gene transfer by conjugation between *Y. pseudotuberculosis* and *Y. pestis* has also been demonstrated (Lawton et al., 1968a).

R factors have been transferred to *Y. pestis* and *Y. pseudotuberculosis* (Ginoza and Matney, 1963) and to *Y. enterocolitica* (Knapp and Lebeck, 1967). Wild strains of *Yersinia* carrying R plasmids (Cornelis et al.,

1973; Kanazawa et al., 1979) appear to be rare. This could be explained, at least for *Y. enterocolitica*, by the presence of a retriction-modification system (Cornelis and Colson, 1975). Metabolic plasmids coding for lactose and raffinose fermentation have been described in *Y. enterocolitica* (Cornelis et al., 1976).

Other plasmids related to various virulence tests (Ca^{2+} dependency, autoagglutination, lethality for mice and gerbils, Sereny test) have been demonstrated in *Y. pestis* (Ferber and Brubaker, 1981), *Y. pseudotuberculosis* (Gemski et al., 1980b) and *Y. enterocolitica* (Zink et al., 1980; Gemski et al., 1980a). These plasmids of 40–48 megadalton molecular weight constitute a family of related plasmids (Portnoy et al., 1981; Ben Gurion and Shafferman, 1981). *Y. pestis* and *Y. pseudotuberculosis* have never been found to be lysogenic, whereas of 1252 strains of *Y. enterocolitica* studied, 86.4% were lysogenic when grown at 25°C but not at 37°C (Nicolle et al., 1973). Phages active on *Y. pestis* and *Y. pseudotuberculosis* have been described (Gunnison et al., 1951; Girard, 1953), but they are not host-specific and are used only for presumptive bacteriological diagnosis. Coliphages T_2, T_3 and T_7 are also active on *Y. pseudotuberculosis* and *Y. pestis* (Hertman, 1964; Ackerman and Poty, 1969). A phage typing system, useful in epidemiology, has been developed for *Y. enterocolitica* (Nicolle et al., 1973): strains of *Y. enterocolitica* serogroup 03 are associated with phagovar VIII in Europe, IXa in the Republic of South Africa, and IXb in Canada.

Strains of *Y. pestis* produce a bacteriocin active on *Y. pseudotuberculosis* Ben Gurion and Hertman, 1958). This was named Pesticin I by Brubaker and Surgalla (1962) after they detected a second bacteriocin (Pesticin II) which was produced by *Y. pestis* and *Y. pseudotuberculosis*. Pesticin I is also active on certain strains of *E. coli*. *Y. pestis* strains that produce Pesticin I also elaborate a fibrinolytic factor and a coagulase (Brubaker, 1972). A bacteriocin-like activity associated with the presence of phage tails has been ascribed in *Y. enterocolitica* (Nicolle et al., 1973). *Y. intermedia* produces a bacteriocin-like substance at 25°C but not at 37°C that is active on certain strains of *Y. enterocolitica*, *Y. intermedia*, *Y. frederiksenii* and *Y. kristensenii* (Botonne et al., 1979).

The antigenic structure of *Yersinia* species is complex, but some antigens are shared by *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolica*. The common enterobacterial antigen has been found in all species investigated (Le Minor et al., 1972a; Maeland and Digranes, 1975). The Fraction 1 envelope antigen (F1) of *Y. pestis* is best produced when cultures are incubated at 37°C on protein-rich media (Fox and Higuchi, 1958). This antigen is heat-labile (10 min at 100°C), water-soluble, and contains a carbohydrate protein (F1A) and a carbohydrate-free protein (F1B). Passive hemagglutination with F1 antigen is used for serologic surveys in plague foci. The presence of this antigen has also been demonstrated in *Y. pseudotuberculosis* (Quan et al., 1965). V and W antigens expressed by virulent strains of *Y. pestis* cultivated at 37°C appear to be related to the presence of a 45 megadalton plasmid (Ferber and Brubaker, 1981; Ben Gurion and Shafferman, 1981). Production of plasmid-mediated V and W antigens has also been described in *Y. pseudotuberculosis* Gemski et al., 1981b) and in *Y. enterocolitica* (Gemski et al., 1981a). The somatic antigen of *Y. pestis* is rough (R antigen) and therefore no serogroups have been described in this species. This R antigen is also present in *Y. pseudotuberculosis* (Thal and Knapp, 1971). In addition, *Y. pestis* and *Y. pseudotuberculosis* share at least 11 out of 18 antigens studied by Lawton et al. (1960). *Y. pestis* and *Y. enterocolitica* express common protein antigens (Barber and Eylan, 1976). The antigenic scheme for *Y. pseudotuberculosis* (Thal and Knapp, 1971) comprises 6 main thermostable serogroups (I to VI) with subgroups (A, B, 2 to 15), and 5 thermolabile flagellar H antigens (a to e). Antigenic relationships have been demonstrated between *Y. pseudotuberculosis* (serogroups II, IV, IVA and VI) and the following organisms: *Salmonella* serogroups B and D, *E. coli* serogroups 017, 055 and 077, and *Enterobacter cloacae* (Knapp, 1968; Mair and Fox, 1973).

Wauters et al. (1972) described 34 different O antigen and 20 H antigen serogroups in *Y. enterocolitica*. This classification included some serogroups defined by strains belonging to *Y. intermedia* (017) and *Y. kristensenii* (011, 012, 028). Nevertheless, these serogroups are useful epidemiological markers. Crossreactions occur between *Y. enterocolitica* serogroup 09 and *Brucella* species (Hurvell and Lindberg, 1973), and between *Y. enterocolitica* serogroup 012 and *Salmonella* factor 047 (Le Minor et al., 1972b).

Yersinia species are susceptible in vitro to the following antimicrobial agents: tetracycline, chloramphenicol, aminoglycosides (streptomycin, gentamicin, kanamycin and neomycin), sulfonamides (alone or in combination with trimethoprim), and nalidixic acid. They are susceptible to some degree towards colistin and are resistant to erythromycin and novobiocin. *Y. pestis* and *Y. pseudotuberculosis* are usually susceptible to β-lactam antibiotics but their susceptibility to penicillin is in the range of sensitive to intermediate. Resistance to ampicillin (Borowski and Zaremba, 1973) and to streptomycin (Kanazawa and Ikemura, 1979) has been described for *Y. pseudotuberculosis*, *Y. enterocolitica*, *Y. intermedia* (Botonne, 1977). *Y. frederiksenii* and *Y. kristensenii* probably are resistant to penicillin and slightly susceptible or resistant to other β-lactam antibiotics (ampicillin, carbenicillin, cephalothin) (Bercovier et al., 1979). The level of resistance is strain-dependent (Zaremba and Aldova, 1979) and temperature-dependent (Chester and Stotzky, 1976). *Y. enterocolitica* strains produce both a constitutive β-lactamase (active on ampicillin, carbenicillin, penicillin and cephalosporins) and an inducible β-lactamase (active only on cephalosporins and penicillin) (Cornelis and Abraham, 1975). *Y. enterocolitica* strains that are resistant to tetracycline, chloramphenicol, streptomycin and kanamycin have been reported (Zaremba and Aldova, 1979).

Y. pestis is the causative agent of plague. Plague is primarily a disease of wild rodents. *Y. pestis* is transmitted among wild rodents by fleas, in which the bacteria multiply and block the esophagus and the pharynx. The fleas regurgitate the bacteria when they take their next blood meal and transmit the disease to man if no other hosts are available. Infective flea bites produce the typical bubonic form of plague in humans. *Y. pestis* multiplies intracellularly in the host and proceeds through the lymphatic system. The lymph nodes near the flea bite are the first to become inflamed and enlarged, constituting the bubo. The evolution of the infection is usually so rapid that no characteristic lesions are found in the spleen or liver at autopsy. If not treated, the disease evolves in 5–10 days to septicemia and sometimes to a secondary pneumonia. From the latter situation, primary pneumonic plague can spread by means of droplets from man to man. In this clinical form death generally occurs in less than 4 days. *Pestis minor* cases, in which the bacteria remain self-limited in buboes followed by self-cures have been described in endemic plague areas (Pollitzer, 1954).

The virulence of *Y. pestis* is associated with the presence of 6 factors (Surgalla et al., 1968): (a) the ability to produce the F1 antigen, (b) the V,W antigens (associated with Ca^{2+} dependency, growth inhibition on oxalate medium, and autoagglutination when cultures are incubated at 37°C), (c) a pigment (incorporation of Congo red dye or hemin), (d) Pesticin I, (e) a toxin (the "murine toxin," whose activity is not clearly established), and (f) the ability to synthesize purines. The LD_{50} dose for mice inoculated with strains expressing the aforementioned virulence factors is 1–10 organisms. Avirulent strains of *Y. pestis* never produce V,W antigens except in the case of the vaccine strain EV76, whose attenuated virulence has resulted from a mutation in its iron metabolism. Virulent strains and the EV76 strain harbor a 45 megadalton plasmid. In contrast to the V,W antigens, the lack of any of the other virulence factors does not completely abolish the virulence of *Y. pestis* strains.

Y. pseudotuberculosis is responsible for epizootics in nearly all animal species, especially in rodents. Animals are usually contaminated by the oral route and, after 1 or 2 weeks of incubation, the bacteria are found in the mesenteric lymph node. The main symptoms are mesenteric adenitis and chronic diarrhea. The infection evolves either to a self-cure or to a fatal septicemia. *Y. pseudotuberculosis* is an intracellular parasite and, like *Y. pestis*, reaches the lymphatic system. At autopsy, caseous lesions are found in the Peyer's patches, the mesenteric lymph node, the spleen and the liver. Humans orally contaminated by *Y.*

pseudotuberculosis develop either a mesenteric adenitis which simulates an acute appendicitis, or, in the compromised host, a severe septicemia. *Y. pestis* and *Y. pseudotuberculosis* appear to share at least two virulence factors: the F1 antigen and the V,W antigens.

Y. enterocolitica has been recognized as pathogenic for chinchillas, hares, monkeys and humans. The pathogenicity for animals is similar to that of *Y. pseudotuberculosis*. In children, *Y. enterocolitica* is responsible for acute adenitis simulating appendicitis, and also for terminal ileitis with diarrhea. In adults the main clinical forms of infection are arthritis, septicemia and erythema nodosum. The infection is probably acquired orally, and the bacteria multiply first in the Peyer's patches of the host. Then, depending on its serogroup and on the presence of a plasmid associated with virulence (V,W antigens expressed or positive autoagglutination test), the bacteria remain localized in the gut (ileitis) or invade lymphatic organs (mesenteric adenitis) and eventually reach the blood circulation (septicemia). Arthritis is caused mainly by serogroup 09, which has antigens in common with *Brucella*. This symptom is closely associated with the presence of the histocompatibility antigen HLA-27 in man (Bottone, 1977; Mollaret et al., 1979). Production of a heat-stable enterotoxin (ST) resembling *E. coli* ST (Okamoto et al., 1981) has been demonstrated in vitro (Pai and Mors, 1978), but its role in pathogenicity is not clear: *Y. enterocolitica* strains do not produce ST when incubated in vitro at temperature above 30°C, and no direct proof of production of ST in vivo has been reported.

Virulent strains of *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica* rapidly become avirulent when subcultured on nutrient media incubated at 37°C. This is a result of the loss of the virulence plasmid associated with Ca^{2+} dependency and production of the V,W antigens. Cross-immunity among these three species has been demonstrated (Thal, 1973; Alonso et al., 1978). Human chemoprophylaxis with sulfonamides, vaccination, and the spreading of insecticides and rodenticides are the suggested measures for controlling plague. The drugs of choice for treatment for all *Yersinia* infections are streptomycin, the sulfonamides, chloramphenicol and the tetracylcines.

The pathogenicity of *Y. intermedia*, *Y. kristensenii* and *Y. frederiksenii* in man and animals is not clearly established. They all behave more like opportunistic pathogens than true pathogens (Bottone, 1977; Bercovier et al., 1978). ST-producing strains of these three species have been described (Kapperud, 1980), but their clinical significance is still unknown. *Y. ruckeri* is a fish pathogen responsible for red mouth disease, especially in rainbow trout. An inflammation of the mouth and the throat is the main characteristic of the disease which is enzootic (Rucker, 1966). The bacterium is usually isolated from the kidneys of fish undergoing a systemic infection.

The geographical distribution of *Y. pestis* is widespread, and the organism has been isolated from all the continents. Plague is enzootic in Africa (Central, East and South Africa), in North and South America, and in Asia (Southeast Asia, U.S.S.R., Iran). Between epidemics, *Y. pestis* remains localized in definite foci (Balthazard, 1964). It has been isolated from more than a hundred different naturally infected species of rodents, but rarely from predatory animals (carnivores and birds, the latter being resistant to the infection). The spread of plague is usually accomplished by the cycle of rodents to fleas, fleas to rodents. The reservoir of the bacteria is the soil contaminated by infected dead fleas and rodents. The bacteria survive for months in deep burrows. Rodents coming from noninfected areas become infected when they dig burrows in previously contaminated areas (Mollaret et al., 1963). This cycle constitutes the "sylvatic plague." When urban rodents are in contact with rural rodents, the bacteria can spread to humans through flea bites. The epidemiology of plague is linked to the ecology of both fleas and rodents.

Y. pseudotuberculosis is distributed worldwide. It has been found in numerous animal species, especially rodents and birds, in soil, and in man (Wetzler, 1970). Wild animals, which are often asymptomatic carriers, are considered the reservoir of the bacteria. Man and animals are contaminated orally either by direct contact with sick or asymptomatic animals or through food contaminated by the excretions of these animals. The incidence of this infectious disease varies with the seasons and is highest during the cold seasons. *Yersinia* species multiply even at 4°C and therefore have a selective advantage over other bacteria at low temperatures; this explains why *Y. pseudotuberculosis*, *Y. enterocolitica*, *Y. frederiksensii* and *Y. kristensenii* are more frequently isolated from the environment during the cold seasons than during the hot seasons. Human and animal infections follow this seasonal distribution as well.

Y. enterocolitica has been isolated from a wide variety of sources (live and inanimate) in every country in which it has been looked for and probably has a worldwide distribution (Mollaret et al., 1979). Biovar 1 strains are ubiquitous, having been found in a wide range of animals and environmental sources (including foods), whereas other biovars or serogroups are frequently associated with a specific host (Bercovier et al., 1980a): biovar 5 strains have been isolated mainly from hares in Europe; biovar 4, serogroup 03 strains and biovar 3, serogroup 05,27 strains are responsible for most human gastrointestinal infections in Europe, Canada and the Republic of South Africa; serogroup 08 strains are frequently isolated from various syndromes in the United States; serogroup 09 strains are closely associated with human arthritis in Europe.

Y. intermedia and *Y. frederiksenii* have been identified in Europe, America, Australia and New Zealand, Israel and Japan. These two species have been isolated mainly from fresh water and foods and only rarely from nonirrigated soil, man or animals other than fish (Bercovier et al., 1978; Brenner et al., 1980a; Kapperud 1977; Ursing et al., 1980a). *Y. kristensenii* has been found in Europe, America, Japan and Australia. Strains of this species have been isolated mainly from soil, foods, and asymptomatic animals; isolates from other environmental sources and from human infection are rare (Bercovier et al., 1980c).

Y. ruckeri has been encountered only in the United States and Canada. It seems to be a natural component of the fresh water ecosystem. The red mouth disease appears only when fish are exposed to large number of bacteria, as has been shown experimentally (Ross et al., 1966). The disease is usually enzootic and occasionally epizootic in fish hatcheries.

Enrichment and Isolation Procedures

Isolation of *Yersinia* strains from noncontaminated samples (blood, lymph nodes) can be performed by using blood agar or nutrient agar incubated for 48 h at 28°C, or 24 h at 37°C followed by 24 h at room temperature. The isolation of *Y. pestis* from contaminated samples requires inoculation (subcutaneously or percutaneously) of animals (guinea pigs, mice or rats). The organism can be cultured from the spleen, liver or lymphatic nodes of the inoculated animals. All other *Yersinia* species will usually be isolated from stools or food samples by inoculating standard or special selective bile-salt media such as MacConkey agar (Lee, 1977), DCL agar, salmonella-shigella agar, SS-D agar (Wauters, 1973), CAL medium (Dudley and Shotts, 1979), CIN medium (Schiemann, 1979), oxalate medium (Soltesz et al., 1980) and BABY 4 medium (Bercovier, unpublished results). All these media should preferably be incubated for 48 h at 28–29°C or for 24 h at 37°C followed by 48–72 h at room temperature. Recovery of *Yersinia* strains from contaminated samples can be improved by various cold enrichment techniques (Lee et al., 1980; van Pee and Straiger, 1979).

Maintenance Procedures

Stab inoculations of *Yersinia* strains in conventional stock culture media stored in the dark at room temperature or at 4°C provide living cultures for 10 years or more, if the tubes are tightly sealed. Lyophilization and deepfreeze storage in 10% glycerol are suitable preservation techniques. To keep a strain fully virulent, it should never be subcultured at 37°C, but always at 25–28°C.

Procedures for Testing Special Characteristics

Methods to test tetrathionate reduction, tellurite reduction and the type of nitrate reductase have been described or referenced by Bercovier

et al. (1979). The Ca^{2+}-dependency of virulent *Yersinia* strains is evaluated on magnesium oxalate medium* (Higuchi and Smith, 1961), as follows. Inoculate 0.1 ml of a bacterial suspension (10^5 bacterial/ml) onto two plates: one is incubated at 37°C, the other at 26°C. Check colony numbers on the two plates after 2 or 3 days. Colonies growing at 26°C but not at 37°C are Ca^{2+}-dependent. A fully virulent strain should give confluent growth at 26°C, whereas only 10–100 colonies should appear at 37°C. The *autoagglutination test* (Laird and Cavanaugh, 1980) to detect virulent *Yersinia* strains is done by inoculating 10 or more isolated colonies, each one into a pair of tubes (13 × 100 mm) containing 2 ml of RPMI-1640 medium containing 10% fetal calf serum and 25 mM HEPES buffer (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid). One tube is incubated at 37°C, the other at 26°C. After incubation at 26°C for 18 h, virulent colonies give a uniform turbidity; at 37°C a layer of agglutinated bacteria appears at the bottom of the tube and the supernatant remains clear. Avirulent strains give uniform turbidity at both 26°C and 37°C, and rough strains show spontaneous agglutination at both temperatures.

Differentiation of the genus **Yersinia** *from other genera*

Characteristics useful for differentiating *Yersinia* from other physiologically similar genera are listed in Table 5.47.

Taxonomic Comments

The genus *Yersinia* was proposed by van Loghem (1944) in order to separate *Y. pestis* and *Y. pseudotuberculosis* (formerly in the genus *Pasteurella*) from *Pasteurella* species sensu stricto (i.e. *P. multocida*, etc.), from which they differ in their oxidase reaction and in their DNA base composition (Mollaret, 1965). The genus *Yersinia* belongs to the family *Enterobacteriaceae*. *E. coli* tDNA (i.e. the genes coding for transfer RNA) and *Y. pestis* DNA are 63% related (Brenner et al., 1977), a value similar to that found for *E. coli* tDNA and *Hafnia alvei* DNA. All *Yersinia* species express the common enterobacterial antigen. Their physiological characteristics and their fatty acid contents are similar to those of all *Enterobacteriaceae* species. The mol% G + C of *Yersinia* species ranges from 46–50 and is consistent with that for *Enterobacteriaceae* species.

The genus *Yersinia* presently consists of seven different species. On the basis of DNA/DNA hybridization studies, all of these species are more closely related to each other than to any other *Enterobacteriaceae* species (Brenner et al., 1978; Brenner et al., 1980b; see also Table 5.51. The genus *Yersinia* can be considered a very homogeneous taxon.

DNA relatedness among *Yersinia* species is 40% or higher except for *Y. ruckeri* which is at most 38% related to other *Yersinia* species. DNAs of *Y. ruckeri* strains have been shown to be 30% related to *Serratia* species (Ewing et al., 1978). *Y. ruckeri* was included in *Yersinia* because its mol% G + C of 48 is closer to that of *Yersinia* species than to that of *Serratia* species. Because the phenotypic characteristics of *Y. ruckeri* are very different from those of other *Yersinia* species (see Tables 5.48 and 5.49), it might constitute a new genus by itself. Phylogenetic studies would be helpful in clarifying this problem.

Strains of *Y. enterocolitica* belonging to the five different biovars (see Table 5.50), including the metabolically inactive biovar 5 strains, constitute a homogeneous genospecies (Bercovier et al., 1980a). The strains described as *Y. enterocolitica*-like organisms or atypical *Y. enterocolitica* are separated into three different species: *Y. intermedia* (Brenner et al., 1980a), *Y. frederiksenii* (Ursing et al., 1980a), and *Y. kristensenii* (Bercovier et al., 1980c). *Y. frederiksenii* consists of three genetic groups on the basis of DNA/DNA hybridization (Ursing et al., 1980a). For practical reasons, because there are no phenotypic differences among the three genetic groups, only one species has been proposed for the rhamnose-positive strains. More study on phenotypic characteristics is needed in order to separate the three genetic groups.

The DNAs of *Y. pestis* strains, regardless of biovar, and of *Y. pseudotuberculosis* are 90% or more interrelated. This explains the antigenic and biochemical similarities of the two species (Mollaret, 1965). On the basis of DNA data, Bercovier et al. (1980b) proposed that the two species constitute a single species, divided into two subspecies: *Y. pseudotuberculosis* subspecies *pseudotuberculosis* and *Y. pseudotuberculosis* subspecies *pestis*. This proposal was made in order

Table 5.47.
Differential characteristics of the genus **Yersinia** *and other physiologically similar genera*[a]

Characteristics	*Yersinia*	*Hafnia*	*Citrobacter*	*Escherichia*	*Enterobacter*	*Klebsiella*	*Salmonella*	*Proteus*	*Pasteurella*
Oxidase test (tetramethylphenylenediamine)	−	−	−	−	−	−	−	−	+
Colony size greater than 1.0 mm on nutrient agar, 24 h, 37°C	−	+	+	+	+	+	+	+	−
Motility at:									
37°C	−	+	+	D	+	−	+	+	−
25°C	D	+	+	+	+	−	+	+	−
Gas from glucose fermentation	− or W	+	+	+	+	D	+	+	−
Citrate (Simmons'), 37°C	−	−	+	−	+	D	+	D	−
Voges-Proskauer test, 25°C	D	+	−	−	D	D	−	D	−
Lysine decarboxylase	D	+	−	+	D	D	+	−	−
H_2S production (Kligler)	−	−	D	−	−	−	+	D	−
Phenylalanine deaminase	−	−	−	−	−	−	−	+	−
Mol% G + C of DNA	46–50	48–49	50–52	48–52	52–60	53–58	50–53	38–41	40–45

[a] Symbols: see standard definitions; also, W, weak reaction.

*Magnesium oxalate medium: blood agar base (BBL, or any other manufacturer if the Ca^{2+} content of the base is low), 40.0 g; distilled water, 830 ml. Sterilize at 121°C for 15 min and cool to 45°C. From stock solution sterilized by filtration, aseptically add the following ingredients: $MgCl_2$ solution (23.8 g/liter),80 ml; sodium oxalate solution (33.5 g/liter), 80 ml; and glucose solution (180.2 g/liter), 10 ml.

to embrace the available scientific knowledge and to comply with public health requirements.

Ursing et al. (1980b) have shown, on the basis of DNA and physiological data, that *Y. philomiragia* (Jensen et al., 1969) is not related to the genus *Yersinia* and, furthermore, that it is not a member of the family *Enterobacteriaceae*. These authors stated that until a proper assignment is made for this species it should be referred to as the "Philomiragia bacterium."

Acknowledgments

We wish to acknowledge our indebtedness to D. J. Brenner and L. Levy for reviewing this chapter and to R. Siegel for her help in typing, patience and skillful assistance.

Further Reading

Bercovier, H., D. J. Brenner, J. Ursing, A. G. Steigerwalt, G. R. Fanning, J. M. Alonso, G. P. Carter and H. H. Mollaret. 1980. Characterization of *Yersinia enterocolitica sensu stricto*. Curr. Microbiol. *4:* 201–206.

Bercovier, H., H. H. Mollaret, J. M. Alonso, J. Brault, G. R. Fanning, A. G. Steigerwalt and D. J. Brenner. 1980. Intra- and interspecies relatedness of *Yersinia pestis* by deoxyribonucleic acid hybridization and its relationship to *Yersinia pseudotuberculosis*. Curr. Microbiol. *4:* 225–230.

Bercovier, H., J. Ursing, D. J. Brenner, A. G. Steigerwalt, G. R. Fanning, G. P. Carter and H. H. Mollaret. 1980. *Yersinia kristensenii*: a new species of *Enterobacteriaceae* composed of sucrose-negative strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). Curr. Microbiol. *4:* 219–224.

Brenner, D. J., H. Bercovier, J. Ursing, J. M. Alonso, A. G. Steigerwalt, G. P. Carter and H. H. Mollaret. 1980. *Yersinia intermedia*: a new species of *Enterobacteriaceae* composed of rhamnose-positive, melibiose-positive, raffinose-positive strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). Curr. Microbiol. *4:* 207–212.

Brenner, D. J., J. Ursing, H. Bercovier, A. G. Steigerwalt, G. R. Fanning, J. M. Alonso and H. H. Mollaret. 1980. Deoxyribonucleic acid relatedness in *Yersinia enterocolitica* and *Yersinia enterocolitica*-like organisms. Curr. Microbiol. *4:* 195–200.

Carter, P. B., L. Lafleur and S. Toma (Editors). 1979. Third International Symposium on *Yersinia*, Contr. Microbiol. Immunol. Vol. 2, Karger, Basel.

Pollitzer, R. 1954. Plague. W. H. O. Monograph Series No. 22, World Health Organization, Geneva.

Ursing, J., D. J. Brenner, H. Bercovier, G. R. Fanning, A. G. Steigerwalt, J. Brault and H. H. Mollaret. 1980. *Yersinia frederiksenii*: a new species of *Enterobacteriaceae* composed of rhamnose-positive strains (formerly called atypical *Yersinia enterocolitica* or *Yersinia enterocolitica*-like). Curr. Microbiol. *4:* 213–218.

Differentiation of the species of the genus Yersinia

Characteristics useful in differentiating the various species of *Yersinia* are listed in Table 5.48.

List of the species of the genus Yersinia

1. **Yersinia pestis** (Lehmann and Neumann 1896) Van Loghem 1944, 15.^{AL} (*Bacterium pestis* Lehmann and Neumann 1896, 194; *Yersinia pseudotuberculosis* subsp. *pestis* Bercovier, Mollaret, Alonso, Brault, Fanning, Steigerwalt and Brenner 1981, 383.^{VP})

pes'tis. L. noun *pestis* plague, pestilence.

The characteristics are as described for the genus and as listed in Tables 5.48 and 5.49.

Three biovars have been described in relation to the geographical distribution of the organism: (a) biovar *antiqua* produces acid aerobically from glycerol, reduces nitrate to nitrite, does not ferment melibiose, and is found in Central Asia and Central Africa; (b) biovar *medievalis* produces acid from both glycerol and melibiose but does not reduce nitrate to nitrite; it is found in Iran and the U.S.S.R.; and (c) biovar *orientalis* (synonym: *oceanic*) does not produce acid from either glycerol or melibiose but reduces nitrate to nitrite and is distributed worldwide.

Some rare atypical strains positive in their reactions for urease and rhamnose have been reported.

Table 5.48.
Characteristics differentiating the species of the genus **Yersinia**[a]

Characteristics	1. *Y. pestis*	2. *Y. pseudo-tuberculosis*	3. *Y. enterocolitica*	4. *Y. intermedia*	5. *Y. frederiksenii*	6. *Y. kristensenii*	7. *Y. ruckeri*
Motility (25°C)	−	+	+	+	+	+	d
Lysine decarboxylase (Møller)	−	−	−	−	−	−	+
Ornithine decarboxylase (Møller)	−	−	+	+	+	+	+
Urease	−	+	+	+	+	+	−
β-Xylosidase[b]	+	+	−	−	d	−	−
Gelatinase	−	−	−	−	−	−	+
Citrate (Simmons'), 25°C	−	−[c]	−	+	d	−	+
Voges-Proskauer test, 25°C	−	−	+	+	+	−	d
Indole production	−	−	d	+	+	d	−
γ-Glutamyl transferase	−	d	+	+	+	+	+
Acid production from:							
Rhamnose	−	+	−	+	+	−	−
Sucrose	−	−	+	+	+	−	−
Cellobiose	−	−	+	+	+	+	−
Melibiose	d	+	−	+	−	−	−
α-Methyl-D-glucoside	−	−	−	+	−	−	−
Sorbose	−	−	+	+	+	+	−
Sorbitol	−	−	+	+	+	+	−
Raffinose	−	d	−	+	−	−	−

[a] For symbols see standard definitions.
[b] Using *p*-nitrophenyl-β-D-xylopyranoside as substrate.
[c] Strains belonging to serogroup IV are citrate-positive.

Table 5.49.
Other characteristics of the species of the genus **Yersinia**[a]

Characteristics	1. *Y. pestis*	2. *Y. pseudotuberculosis*	3. *Y. enterocolitica*[b]	4. *Y. intermedia*	5. *Y. frederiksenii*	6. *Y. kristensenii*	7. *Y. ruckeri*
Catalase	+	+	+	+	+	+	+
Oxidase	−	−	−	−	−	−	−
Pigment formed	−	−	−	−	−	−	−
Motility, 37°C	−	−	−	−	−	−	−
Methyl red test, 37°C	+	+	+	+	+	+	+
Voges-Proskauer test, 37°C	−	−	−	−	−	−	−
Citrate (Simmons'), 37°C	−	−	−	−	−	−	−
KCN, growth in, 37°C	−	−	−	−	−	−	d
Malonate utilization	−	−[c]	−	−	−	−	−
D-Tartrate utilization	−	−	−				−
Mucate utilization	−	−	−	d	−	−	−
Citrate (Christensen)	−	−	d	d	d	d	+
Nitrate reduced to nitrite	d	+	+	+	+	+	+
Oxidation-fermentation test (Hugh-Leifson)	O/F	O/F	O/F	O/F	O/F	O/F	O/F
D-glucose, gas production	−	−	v and W[d]	v and W	v and W	v and W	v and W
H_2S production (Kligler)	−	−	−	−	−	−	−
Tetrathionate reductase	−	d	d	+	+	d	
Phenylalanine or tryptophan deaminase	−	−	−	−	−	−	−
Arginine dihydrolase (Møller)	−	−	−	−	−	−	−
β-Galactosidase[e]	+	+	+	+	+	+	+
Lipase (Tween 80)	−	−	d	d	d	d	
Deoxyribonuclease	+	d	d	−	−	−	−
Acid production from:							
Glucose, fructose, galactose, ribose, mannose, maltose, trehalose, *N*-acetylglucosamine, mannitol	+	+	+	+	+	+	+
L-Arabinose	+	+	+	+	+	+	−
Glycerol	d	+	+	+	+	+	−
i-Inositol	−	−	+	+	+	+	−
D-Xylose	+	+	d	+	+	+	−
Esculin	+	+	d	+	+	d	−
Amygdalin	−	−	v	+	+	v	−
Arbutin	+	d	v	+	+	v	
Salicin, dextrin	d	d	v	+	+	v	
Lactose	−	−	d	−	d	d	−
Adonitol, erythritol, dulcitol, D-arabinose, L-xylose, methyl-D-mannoside, methyl-xyloside, melezitose, inulin	−	−	−	−	−	−	−

[a] Tests were incubated at 28°C except where indicated, and were read during 3 days. For symbols see standard definitions.
[b] Tests are given for biovars 1 to 4.
[c] Strains belonging to serogroup IV are malonate-positive.
[d] W, weak reaction.
[e] Using *o*-nitrophenyl-β-D-galactopyranoside as substrate.

Y. pestis is the causative agent of plague. The disease can be reproduced experimentally in mice, rats, guinea-pigs and monkeys.

The mol% G + C of the DNA is 46 (T_m).

Type strain: ATCC 19428 (NCTC 5923).

2. **Yersinia pseudotuberculosis** (Pfeiffer 1889) Smith and Thal 1965, 220.[AL] (*Bacillus pseudotuberculosis* Pfeiffer 1889, 5; *Yersinia pseudotuberculosis* subsp. *pseudotuberculosis* (Pfeiffer 1889) Smith and Thal 1965, 220; see Bercovier et al. 1983, 383.)

pseu.do.tu.ber.cu.lo'sis. Gr. adj. *pseudes* false; M.L. fem. n. *tuberculosis* tuberculosis; M.L. gen. n. *pseudotuberculosis* of false tuberculosis.

The characteristics are as described for the genus and as listed in Tables 5.48 and 5.49.

Table 5.50.
Differentiation of the biovars of **Yersinia enterocolitica**

Characteristics	Biovar 1	2	3	4	5
Lipase (Tween 80)	+	−	−	−	−
Deoxyribonuclease	−	−	−	+	+
Indole production	+	+	−	−	−
Nitrate reduced to nitrite	+	+	+	+	−
Acid production from:					
D-Xylose	+	+	+	−	−
Sucrose	+	+	+	+	d
D-Trehalose	+	+	+	+	−

Some freshly isolated strains may require subculturing before expressing their motility.

Strains belonging to serogroup IV are citrate-positive (Simmons') and malonate-positive.

Up to 5% of *Y. pseudotuberculosis* strains have been reported to produce acid from adonitol.

Some strains, mostly of serogroup III, produce an exotoxin that differs from the *Y. pestis* toxin. The biological activity is not well defined.

Y. pseudotuberculosis is a human and animal pathogen responsible for mesenteric lymphadenitis, diarrhea and septicemia. The disease can be reproduced experimentally in guinea-pigs challenged *per os* and in mice. Aureomycin given orally to guinea-pigs induces the disease in healthy carriers.

The mol% G + C of the DNA is 46.5 (T_m).

Type strain: ATCC 29833 (NCTC 10275). This strain belongs to serogroup I.

3. **Yersinia enterocolitica** (Schleifstein and Coleman 1943) Frederiksen 1964, 104.^{AL} (*Bacterium enterocoliticum* Schleifstein and Coleman 1943, 56.)

en.ter.o.co.li'ti.ca. Gr. n. *enteron* intestine; Gr. n. *colon* the colon; Gr. suff. *-iticos* pertaining to; M.L. fem. adj. *enterocolitica* pertaining to the intestine and colon.

The characteristics are as described for the genus and listed in Tables 5.48 and 5.49.

The Voges-Proskauer test is usually positive at 22–28°C and negative at 37°C.

Biovars of *Yersinia enterocolitica* are listed in Table 5.50 and, like phagovars and serogroups, are useful epidemiological tools.

Rare atypical strains that are either positive for their reactions on Simmons' citrate, for acid production from lactose and raffinose (due to a metabolic plasmid), or negative for urease activity have been reported.

When incubated at 20°C, *Y. enterocolitica* strains produce a broad spectrum mannose-resistant hemagglutinin which is lost at 37°C (MacLaglen and Old, 1980).

Y. enterocolitica is responsible for diarrhea, terminal ileitis, mesenteric lymphadenitis, arthritis and septicemia in man and animals. The disease can be reproduced experimentally in mice, gerbils and monkeys.

The species has been isolated from a wide variety of sources in the environment (live and inanimate) including foods and from healthy man and animals.

The mol% G + C of the DNA is 48.5 ± 1.5 (T_m), Bd).

Type strain: ATCC 9610 (strain 161; CIP 80-27). This strain belongs to biovar 1, serogroup 08 and phagover X_2.

4. **Yersinia intermedia** Brenner, Bercovier, Ursing, Aloniso, Steigerwalt, Fanning, Carter and Mollaret 1981, 217.^{VP} (Effective publication: Brenner et al. 1980, 207.)

in.ter.me'di.a. L. fem. adj. *intermedia* intermediate; here it implies that biochemical reactions of this species seem midway between *Y. enterocolitica* and *Y. pseudotuberculosis*.

The characteristics are as described for the genus and as listed in Tables 5.48 and 5.49.

Media with a high bile salt content (0.8%) are inhibitory, especially when incubated at 37°C.

Some biochemial characteristics (citrate utilization; cellobiose, rhamnose and raffinose fermentation) are always expressed at 25–28°C but are inconstant at 37°C.

Either a type A or a type B nitrate reductase is present.

Eight biovars have been described (Brenner et al., 1980a) based on the fermentation of melibiose, rhamnose, α-methyl-D-glucoside, raffinose and on the utilization of citrate (Simmons'). Of the strains studied, 96% are positive for at least four of these five tests.

Y. intermedia has been isolated mainly from fresh water sources, fish, foods, and occasionally from sick and healthy humans.

The mol% G + C of the DNA is 48.5 ± 0.5 (T_m, Bd).

Type strain: ATCC 29909 (strain 3953; Bottone 48; Chester 48; CIP 80-28).

5. **Yersinia frederiksenii** Ursing, Brenner, Bercovier, Fanning, Steigerwalt, Brault and Mollaret 1981, 217.^{VP} (Effective publication: Ursing et al. 1980, 213.)

fred.er.ik.sen'i.i. M.L. gen. n. *frederiksenii* of Frederiksen; named after the Danish microbiologist Wilhelm Frederiksen, who made a substantial contribution to the study of the genus *Yersinia*.

The characteristics are as described for the genus and as listed in Tables 5.48 and 5.49.

This species is composed of three different genetic groups. One group is positive for β-xylosidase and citrate (Simmons'), and the type strain belongs to this group. The other two groups are variable or negative for these tests. More phenotypic studies are needed to differentiate the three groups.

Some strains are able to ferment raffinose and lactose when they harbor a metabolic plasmid.

Y. frederiksenii has been isolated mainly from fresh water sources, fish, foods, and occasionally from healthy or sick man and animals.

The mol% G + C of the DNA is 48 (T_m).

Type strain: CIP 80-29 (strain 6175).

6. **Yersinia kristensenii** Bercovier, Ursing, Brenner, Steigerwalt, Fanning, Carter and Mollaret 1981, 217.^{VP} (Effective publication: Bercovier et al. 1980, 219.)

kris.ten.se'ni.i. M.L. gen. n. *kristensenii* of Kristensen, named after

Table 5.51.
DNA relatedness and divergence in related sequences in the genus **Yersinia**[a]

Source of Unlabeled DNA	1. *Y. pestis*	2. *Y. pseudotuberculosis*	3. *Y. enterocolitica*	4. *Y. intermedia*	5. *Y. frederiksenii*	6. *Y. kristensenii*	7. *Y. ruckeri*
Y. pestis	97(0)	88(0.1)	43				
Y. pseudotuberculosis		92(1)	59(12)	54(12)		50	30(15)
Y. enterocolitica		48(11.5)	96(2.5)	58(11)	60(12.5)	69(9)	30(15)
Y. intermedia		44(12)	59(12)	95(1.5)	61(11)	62(12)	
Y. frederiksenii		44(11)	67(11)	58(12.5)	81(5)	59(10)	
Y. kristensenii		44(11)	70(9.5)	62(12.5)	55(12)	84(4)	
Y. ruckeri		33(13)	30(15)	38			95(0.1)

[a] Data are from Bercovier et al. (1980), Brenner et al. (1976), Brenner et al. (1980), Ewing et al. (1978), and Ursing et al. (1980). Hybridizations were carried out at 60°C. The first number is the average relatedness in per cent of all unlabeled strains with the specific labeled DNA. Homologous reactions are not included in the average. The second number, in parentheses, is the percentage of divergence calculated on the basis of 1% unpaired bases per 1°C decrease in duplex stability.

the Danish microbiologist Martin Kristensen, who first isolated this organism.

The characteristics are as described for the genus and as listed in Tables 5.48 and 5.49.

Growth is delayed (7 days) when cultures are incubated at 41°C and even at 37°C for some isolates.

Some strains utilize citrate (Simmons') after 7 days incubation at 25°C.

Most strains produce a "musty" or "cabbage-like" odor when grown on nutrient agar.

Some strains produce an enterotoxin (ST) when incubated at 22°C and also at 37°C (Kapperud, 1980).

Y. kristensenii strains have been isolated mainly from soil, from various environmental sources (fresh water, foods) and rarely from healthy or sick man and animals.

The mol% G + C of the DNA is 48.5 ± 0.5 (T_m, Bd).

Type strain: CIP 80-30 (strain 105).

7. **Yersinia ruckeri** Ewing, Ross, Brenner and Fanning 1978, 37.[AL]

ruck'er.i. M.L. gen. n. *ruckeri* of Rucker; name after R. R. Rucker, who studied the red mouth disease and its etiological agents.

The characteristics are as described for the genus and as listed in Tables 5.48 and 5.49.

The cells are 1 μm in width and 2–3 μm in length. Filaments can be seen in old cultures (48 h at 22°C).

Colonies on nutrient agar are smooth, circular and slightly raised. Growth is delayed or inhibited on salmonella-shigella agar incubated at 37°C but not at 22°C.

Corn oil is hydrolyzed when the test is performed at 22°C but not at 37°C.

Y. ruckeri is one of the agents responsible for red mouth disease in rainbow trout. The disease can be transmitted experimentally from fish to fish. The organism has been isolated only in North America.

The mol% G + C of the DNA is 48 ± 0.5 (Bd).

Type strain: ATCC 29473.

Species Incertae Sedis

Yersinia philomiragia Jensen, Owen and Jellison 1969, 1237.[AL]

phi.lo.mi.ra'gi.a. Gr. adj. *philos* loving; M.L. n. *miragia* plural of Latinized English word *mirage*; *philomiragia* loving mirages, because of the mirages that are seen in the area where the isolations of this species were made.

A fastidious organism pathogenic for muskrats. Because of a morphological resemblance to *Y. pestis* and some degree of DNA relatedness to *Y. pestis* (Ritter and Gerloff, 1966), the organism was assigned to the genus *Yersinia*. Later studies indicated, however, that no significant DNA relatedness occurred between *Y. philomiragia* and other *Yersinia* species, other *Enterobacteriaceae* or *Pasteurella multocida* (Ursing et al., 1980b). The strains that have been investigated are phenotypically similar to one another and form a homogenous DNA relatedness group (Ursing et al., 1980b). Until an appropriate genus assignment can be made, it is recommended that the organism be referred to as the "Philomiragia bacterium."

Motility is negative at both room temperature and 36°C.

Strains of the species give positive tests for the following reactions (36°C): catalase, indole production, gelatin hydrolysis, Voges-Proskauer test, and acid production from D-glucose, maltose and sucrose. Reactions for the following tests differ among strains: β-galactosidase and acid production from D-fructose and galactose. The following reactions are negative: oxidase, nitrate reduction to nitrite, H_2S production, urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, citrate utilization (Simmons'), esculin hydrolysis, and acid production from L-arabinose, L-rhamnose, D-raffinose, lactose, D-mannitol, D-sorbitol, *i*-inositol and salicin. Gas is not produced from carbohydrate fermentation.

First isolated in 1959 from a dead muskrat found in a marshy area at the Bear River Migratory Bird Refuge in northern Utah. Other strains have been isolated from water in the same area.

Type strain: ATCC 25015.

Other genera of the family **Enterobacteriaceae**

JOHN J. FARMER III

The previous sections describe the genera of *Enterobacteriaceae* which have been known and thoroughly studied for many years and whose generic names are familiar to most microbiologists. A number of other genera in the family, however, have received little attention: *Obesumbacterium* and *Xenorhabdus* because of their limited ecological niche, *Kluyvera* because of its poor recognition by the scientific community, and *Rahnella*, *Tatumella* and *Cedecea* because of their newness.

The purpose of this section is to acquaint the reader with the limited information about these new genera. For obvious reasons, the material presented cannot be as complete as that given for the other genera in the family. Over the last few years, the Enteric Laboratories at the Centers for Disease Control (CDC) has characterized isolates of all these new genera. This section will summarize the material in the literature about the new groups as well as our own findings.

Unless otherwise stated, all data are based on cultures studied by the Enteric Laboratories, CDC. Biochemical testing was by the method of Edwards and Ewing (1972), which has been updated (Farmer et al., 1980). Incubation was at 36 ± 1°C except for cultures of *Xenorhabdus*, which grew poorly or not at all at 36°C and which were tested at 25°C (Table 5.52). All enzyme names should be understood to be in quotation marks since actual enzyme assays with cell-free extracts were not done.

Stocks cultures of the six genera were prepared and stored in the same way as other *Enterobacteriaceae*. Cultures were preserved by two methods. In method 1, growth was taken from a trypticase soy agar plate (or any agar medium that allows optimum growth) and a heavy suspension was made in 10% w/v skim milk. This was "quick frozen" in a beaker of 95% ethanol (kept in the −70°C freezer) and then stored at −70°C. In method 2, cultures were inoculated into a solid or semisolid medium (100-× 13-mm screw-cap tubes) such as trypticase soy agar, trypticase soy semisolid (0.4% agar) or blood agar base, incubated 1–2 days until growth was obvious, sealed with a number "000" white rubber stopper and stored in the dark at room temperature. This latter stock culture is called the "working stock" and the −70°C culture is called the "freezer stock." Almost all *Enterobacteriaceae* survive well with both methods, except *Tatumella* cultures which may die when only method 2 is used. Important cultures of *Enterobacteriaceae* are preserved by both methods, but routine cultures are stocked only by method 2. *Tatumella* stocks are done both ways.

Genus **Obesumbacterium** *Shimwell 1963, 759*[AL]

O.be'sum.bac.te'ri.um. L. neut. adj. *obesum* fat; L. neut. n. *bacterium* rod; M.L. neut. n. *Obesumbacterium* a fat, rod-shaped bacterium.

Pleomorphic rods 0.8–2.0 μm in diameter, 1.5–100 μm in length (short, "fat" rods predominate when grown in beer wort with live yeasts, long pleomorphic rods usually predominate when grown in most bacteriological media), conforming to the general definition of the family *Enterobacteriaceae*. **Nonmotile.** Facultatively anaerobic. Very slow growing, forming colonies less than 0.5 mm in diameter on